GENETIC NEUROLOGY

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Problems of the development, growth, and regeneration of the nervous system and of its functions

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PAUL WEISS

Editor



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FOREWORD

THE days of March 21–25, 1949, during which the International Conference on the Development, Growth, and Regeneration of the Nervous System was held at the University of Chicago under the sponsorship of the International Union of Biological Sciences, may at some future time be remembered as the birth date of a new biological discipline—"*genetic neurology*"—birth date in the sense that an organism, which had for some time been in the making, came to light, gave evidence of its vitality, acquired a name, and set out for a career of its own. Neuroanatomy, neurophysiology, and neuropsychology deal essentially with the nervous system in its final mature state. Genetic neurology encompasses all those processes that lead up to that state ("neurogenesis"), maintain it in its integrity, and restore it after disruption. The attribute "genetic," accordingly, refers to "genesis," i.e., development in the widest sense, and not to "genetics" in the narrower modern sense of "inheritance," although it naturally includes the latter. Genetic neurology is a stream with many tributaries. Embryology, histology, physiology, pathology, neurology, psychology, biochemistry, and others have produced much valuable source material. Yet their confluence is a new development. Not until the formerly isolated lines were brought to meet on common ground was there any realization of how many interests they truly shared and how much more profitably they could proceed by com- [illegible]

[illegible] attempt to bring together, compare, and order from a common perspective the many scattered fragments of information on the development and restitution of the nervous system. The task of the conference was one of synthesis. As the meetings proved, it was a most timely one.

The [illegible]

[illegible] provided additional travel funds for participants from abroad. Sincere thanks are due to both these organizations for their assistance. The contribution of the *University of Chicago*, which acted as host and placed its facilities at the disposal of the conference, is also gratefully acknowledged. Most efficient secre-

tarial and technical assistance were rendered by Miss Catharine Overton and Mrs. Margaret W. Cavanaugh.

Since this conference may set a pattern for future similar ones, it is perhaps of value to add a few remarks about its organization and conduct. The speakers were chosen with a view to having the widest possible variety of pertinent fields represented by the most outstanding experts available. To emphasize the international scope, geographical representation was made as broad as was feasible within the limited means. The list of participants following this Foreword will indicate the extent to which these goals have been accomplished. It was most gratifying to have one of the classical masters of nerve histology, Jan Boeke, come to the conference, unmindful of the strains of a hurried trans-Atlantic air trip. It was a privilege to count among the members one of the earliest leaders in experimental embryology and the tissue culture of neurons, Warren Lewis. But it was a grave disappointment that three of the foremost pioneers in genetic neurology, R. G. Harrison, C. J. Herrick, and S. R. Detwiler, could not attend because of conflicting engagements.

On the whole, the old and the new, the descriptive and the analytical, the physicochemical and the organismic, the structural and the physiological, the theoretical and the practical, blended into a harmonious fabric. The procedure of the conference was dictated by its purpose, which was not at all to arrive at formal conclusions but primarily to compare experiences, clarify ideas, scrutinize current notions, remove inconsistencies, sort knowledge from conjecture, and, above all, point up the vast areas of ignorance in need of elucidation. The prevailing spirit was one of informal exploration rather than dogmatic presentation. Accordingly, a somewhat novel technic was adopted. No formal papers were required or read. All members were assumed to have some degree of familiarity with the basic facts and the central issues. More intimate acquaintance grew by a mutual give-and-take, as the discussion went along. Yet, in order to avoid the danger of dissolution inherent in free and rambling conversation, each session was assigned a circumscribed topic on which the discussion had to focus. For each session a panel of four members was appointed whose specialties came closest to the issues to be discussed. At the beginning of each session, the chairman would keynote the chosen topic and outline the range of subjects to be dealt with. The panel of specialists would then elaborate the points raised, and gradually the rest of the group would enter the discussion. Motion pictures and illustrative slides were shown in the proper context. The results

of each session were summarized and reviewed in a final concluding session to which the public was admitted.

Only at the end of the conference were all participants invited to produce written accounts of those aspects of the proceedings closest to their individual interests. Being written after, rather than before, the conference, these accounts could thus draw upon its lessons and attest to the benefits derived from the joint discussions.

Thus originated the collection of essays presented in this little volume. They vary considerably as to form, scope, and content, but this variety is a true reflection of the character of the conference. To obliterate such diversity and to force the

eliminate occasional overlap and repetition among individual articles; to pass editorially on the pertinence of facts, expressions, conclusions, priorities, quotations, and bibliographic citations; or even to affix cross-references pointing to positive or negative correlations among related statements of different authors. The order in which the articles are arranged is likewise arbitrary, except for a vague attempt to link them in some logical sequence.

This volume is as much a review of the native endowment which "genetic neurology" has received from the splendid past contributions of its parent sciences as it is a preview of the large task ahead which it faces on its own. It will be evident that this task can never be accomplished in emancipated isolation but only in harmonious collaboration with all the members of the family of neurological—indeed, with the whole tribe of biological—sciences. The birth of "genetic neurology" marks the establishment not of one more division in an already overfragmented science but, on the contrary, of new organic bonds among the isolated fragments. Not fragmentation, but integration, is its aim.

The fact that the conference was held on an international scale expresses the conviction that this integration rightfully ignores and transgresses boundaries not only between disciplines but between nations and countries as well. Some sciences can perhaps make a more plausible claim for the need of international collaboration, by stressing the world-wide extent of their subject matters, such as oceanography, meteorology, and astronomy. We could rationalize our case by pointing to the overriding importance of the nervous system, whose constructive utilization benefits, but whose misappropriation may doom, humanity. But infinitely more important is the fact that no

such rationalization on purely utilitarian grounds was needed. Quite aside from all practical benefits, the greatest value of such international gatherings lies in the profession and reaffirmation of the unity, universality, and indivisibility of scientific truth and, above all, in the demonstration that scientists are willing to practice what they profess.

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UNIVERSITY OF CHICAGO
May 1950

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CONTENTS

AN INTRODUCTION TO GENETIC NEUROLOGY[1]

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PREAMBLE

THE function of the chairman of a conference is to serve in the catalytic role of moderator. As organizer and chairman of the Conference on the Development, Growth, and Regeneration of the Nervous System my primary task was, therefore, to put the problems in focus and steer the discussion. The unique pattern of publication of the proceedings, however, according to which each participant had to write a contribution in retrospect, implies an obligation to record my own version of the content and achievements of the conference. This can best be done by following the topical organization of the conference [illegible]

[illegible]

random sample of genetic neurology, with a running commentary. Familiar facts will be referred to without further documentation. Items discussed in greater detail in other chapters of the book will be so identified. Many a minor problem, neglected and ill explored in the past, will be accorded more attention and space than some of the major problems on which more adequate information is available. Such arbitrariness of selection, together with the pardonable temptation to illustrate points at issue by examples from one's own experimental experience, is bound to distort the proportions of the total picture. Yet, since the other articles are similarly slanted, and quite deliberately and desirably so, from other angles, their totality ought to present a fairly sound perspective.

If [illegible]
cent [illegible]
posi[illegible] complex nature of the processes involved. As we now view it, development is an assembly-line process in which

[1] Original research referred to in this article has been aided by the Wallace C. and Clara A. Abbott Memorial Fund and by a research grant from the National Institute of Health, Public Health Service.

countless component events are brought together in orderly patterns in regular succession and are interwoven with one another by innumerable specific interactions (89). Thus the closely knit fabric of any organ, structure, or function, which in its mature stage impresses us by its purposeful unity, when traced back in its developmental history resolves itself into numerous separate threads of different origins, different characters, and different dependencies. In this sense, each component process contributes a share to the final assembled product, but the unity of the assemblage is an emergent unity. Although all developmental processes are under some common control from the very first, the composition and the grouping of the operative units change progressively as cell groups give up old and enter new associations, and the various organs acquire their final identity and functional adequacy only by degrees through the co-ordinated commingling and interplay of originally unrelated contributions.

This conclusion of developmental theory finds a natural corollary in the demonstration by genetic theory that any single terminal character may depend on any number of genes and, conversely, any one gene may affect any number of terminal characters. However, while current genetic theory favors the view that all primary gene action is of a single kind, namely, control of enzymatic reactions, developmental theory proves that, at least by the time the various morphogenetic assembly lines are in operation, both the source materials and the tools are of a wide diversity, much as the materials and manufacturing procedures in the making of, let us say, an automobile vary from step to step. The complex engineering performances of technology are a much more pertinent model of the nature of morphogenesis than are the more elementary phenomena dealt with in basic physics and chemistry.

Viewed in this light, the mature nervous system is the terminal product of many convergent chains of processes with cellular contributions from many sources, with incessant interactions among the units and between the units and their environment. In studying the nature of these interactions, we encounter the widest variety of biophysical and biochemical phenomena, with electrical, chemical, thermal, and mechanical factors participating in various combinations. If in some not too remote past the question as to whether a given component of nerve development was exclusively an electric or a chemical or a mechanical phenomenon was pardonable on grounds of a general lack of familiarity with the principles of development, the

same question repeated today would be a sign of lack of comprehension, if not of disregard, of known facts. The oversimplified past theories of nerve development ("electric" or "chemical," etc.) were useful historically as stopgap measures. However, if perpetuated, they will become an intolerable drag on the progress of knowledge, since in their failure to formulate the real problems and in glossing over the complexities of the latter, they hide, rather than reveal, practicable lines of approach It is not so much that they obstruct experimental analysis as that they divert it to rather fictitious tasks.

Consequently, in order to be realistic in our research, we must accept and face the assembly-line character of the nervous system, the multiplicity and heterogeneity of agents that enter into its manufacture, and give up the hope of embracing the whole process in a single, simple formula. We must take the complex fabric as we find it; try to resolve it into its constituent threads, that is, individual chains of causal linkages, and determine their mode of action without the narrow preconception that they must all belong to a single category. Perhaps it has been the most important result of this conference that it engendered and reinforced such an attitude of sober analytical realism. It is remarkable how grandiose concepts often melt away when brought face to face with hard-and-fast facts which they were purported to explain and how the removal of a verbal smoke screen opens exciting new vistas for research. In this spirit of analytical dissection, we shall now proceed to single out a few of the main contributory processes to the development of the nervous system in the order in which they were taken up by the conference.

The six main sessions of the conference were devoted to six relatively separable topics, as follows: (I) the differentiation of the central nervous system and of its units; (II) the outgrowth of nerve fibers and the establishment of nerve connections; (III) the growth and trophic relations of neurons; (IV) the regeneration of nerves, including its implications for the clinical problems of nerve repair; (V) the problems of specificity and selectivity in the development and operation of the nervous system; and (VI) the development of behavior and its neural foundations.

I. NEURON DIFFERENTIATION

The composition and size of any organ [illegible]
[illegible]
[illegible] include migrations, spatial re-

grouping, recruitment of cells from the surroundings, growth, proliferation, loss of cells by destruction or emigration, and differentiation.

A. ALLOCATION

In the nervous system the initial allocation is under the control of influences generally referred to as "inductions" and extensively studied in experimental embryology (69). The allocations seem to bear, from the very first, certain general regional characteristics, which, since they can express themselves in atypical locations, are evidently manifestations of early qualitative differences among different primordial groups of nerve cells (see below, Sec. IF). The early neural system consists of a number of interacting fields (17, 54) which operate in the manner of morphogenetic fields in general (89), that is, by outlining their respective domains through competitive interaction and by initiating further diversifying processes, or subfields, within their respective territories. Local processes thus become progressively more restricted, more firmly fixed, and more autonomous. Depending on how far this course has advanced, injuries to the embryonic nervous system are either wholly or partially or not at all reparable. Many abnormalities of accidental, experimental, or genetic origin, such as cyclopia (2), anophthalmia (14), anencephaly (134), etc., are explicable in reference to the time scale of development, with its progressive loss of regulatory faculties.

B. TRANSFORMATIONS

The early transformations of the neural system are brought about mainly by shifts of cells and cell complexes and changes of mechanical configuration, while growth plays only a subordinate role (27). The mechanics of neurulation are still not yet fully understood, but the formative forces seem to be intrinsic to the neural plate (9), perhaps aided by the differential composition of the media along its inner and outer surfaces. Changes in cell adhesivity (8), as well as the contractility of a surface coat (47), seem involved. Fluid secretion into the central canal and the ventricles (80) serves to keep the brain walls from collapsing, and postero-anterior propulsion of this cerebrospinal fluid by the cilia of the canal may add to the necessary turgor. The mechanics of the formation of fissures, folds, and evaginations in the early brain are not yet well understood but may be due in large measure to mechanical yielding of weaker or more pliable portions of the wall, which expands faster than the capacity of the skull (15). Evidently, the pattern of lines of least resistance along which these

caveins occur is the systematic result of previous developmental events, and its causation, in turn, remains to be explained.

The slit-shape of the central canal depends on the presence of the notochord (37) and is due to the fact that a rigid fiber system spanning the ventral thickness of the neural plate remains inserted on the notochord as a hinge about which the lateral halves fold upward (110). The tangential stresses to which this median strip of the plate is subjected in the folding process are presumably responsible for a corresponding ultra-structural pattern running crosswise in the floor of the tube as a later guide for the ventral commissures (see Sec. II).

Fibers laid down along the surfaces of the original medullary cells, hence radially oriented, are evidently preferential guide lines for the displacement of the cells of the tube. Consequently, all intrinsic growth will lead to expansion in the radial direction. The embryonic tube seems to have little intrinsic tendency to grow in length. Elongation must, therefore, be produced by outside forces, the active agent being presumably the notochord (39, 40). Its elongation confers passive stretch upon the adhering neural tube. Very likely these longitudinal stresses in the margin of the tube furnish, at the same time, the structural foundation for the later longitudinal fiber-tracts.

The spinal ganglia arise as an unsegmented mass, moving down from the neural crest on either side of the cord. The cell columns tend to break up into clusters, but the segmental regularity of this subdivision in normal development is imposed upon them by the segmentation of the surrounding mesoderm (44, 19).

These are merely a few examples of steps in the morphogenesis of the central system. If some of them seem crudely mechanical, it must not be forgotten that they only lay the foundation for many much subtler events to follow and, *moreover*, that the mechanical events in themselves are conditioned by the differential behavior of cell groups governed by subtle biochemical and colloid-physical differences.

C PROLIFERATION

From its early transformations the nervous system emerges endowed with differential *properties which* soon express themselves in differential proliferation, migration, growth, and differentiation, elaborating further the initial differences established in the preceding stages of allocation and transformation The nature and causes of these differentials are *still almost wholly* obscure. Transplantation and defect experiments have revealed at least the time when certain differences arise and the extent to which they are based on properties

intrinsic to the cell groups concerned and on properties of the cellular environments.

The total size of any given central district is the resultant of the following factors: the proportion of cells in proliferative activity, the rate of their multiplication, the duration for which they continue to proliferate, the enlargement of cells without further division, the immigration of cells from the vicinity, the loss of cells, and the differentiation of nerve fibers. The classical experiments of Detwiler (20) have shown that the quantitative development of a nerve center is determined both by its own growth potential and by extraneous influences emanating partly from other central regions and partly from the periphery. But it has remained for the painstaking research of Hamburger and his collaborators to reveal the full complexity of these interrelations and to begin to unravel them. For details we refer to the comprehensive article by Hamburger and Levi-Montalcini in this volume.

The factors maintaining mitotic activity in characteristic distributions are practically unknown. The concept of mitogenetic radiations (28) is generally regarded as unsubstantiated. We have recently discovered that nervous substance contains a diffusible, strongly mitogenetic principle not found in most other organs (55). It will be interesting to explore the relations of this agent to the proliferative activity of the nervous system itself. The only clue regarding the mitogenetic stimuli in the nervous system is the preferential confinement of mitoses to the lining of the central canal and ventricles. The fact that, after injury to cord or brain, cells of the mantle promptly enter into mitosis (21, 33) proves that mantle cells are not incapable of dividing but normally fail to divide merely because of their insulation from the mitotic stimulus, which after injury becomes available to them.

D. MIGRATION

The fact that cells of the central nervous system migrate extensively has long been recognized. Ontogenetic movements recapitulating phylogenetic shifts have been described and labeled as "neurobiotaxis"; but the factors at work have not yet been elucidated. We are faced with essentially the same problems as those presented by the outgrowth of cell processes (see further below). The radial migration of the cells of the neural tube is clearly correlated with the pres-[illegible] constituted by the pri-[illegible] than a [illegible] ay permits movements in opposite directions, and, since both have been ob-

served (see article by Hamburger and Levi-Montalcini), the question remains as to which cells move in one sense and which in the other. Cells first scattered at random gradually accumulate in specific locations, as, for instance, in the formation of the columns of the ventral horns or the various neatly spaced strata of the brain, which adds to the problem of orientation that of the termination of the various movements. Why do cells aggregate at certain sites and not at others? Moreover, since the cells of a given nucleus or a given stratum eventually exhibit common functional properties, the question arises as to whether those properties were imposed upon them by local factors of the zone they occupy or whether they had already been differentiated when they left their sources and merely become selectively attracted or selectively retained in the appropriate environments. (This problem of the origin of specificity of neurons will be taken up later.)

To judge from observations in tissue culture, cell migration is sometimes merely simulated by the fact that the nucleus shifts its position along the drawn-out cell processes without the latter's changing their locations (45). Such a change of nuclear position within a neuron is obviously not the same thing as the displacement of a whole neuron.

For the study of these problems, the derivatives of the neural crest may be more suitable than the intra-central neuroblasts. It has been shown, for instance, for the pigment-producing components of the neural crest that their *migratory urge*, direction of migration, and final localization are determined by separate sets of factors (74); by analogy, we may assume that the factors causing the ganglionic elements of the crest to migrate are also different and less specific than those that cause them to settle at specified sites as spinal ganglia, sympathetic ganglia, and myenteric plexus. Noting further the regular association of sheath cells with nerve fibers (see article by Speidel), it is difficult *to escape* the conclusion that these various derivatives of the crest are gathered into their final locations [illegible] mechanical [illegible] environm[illegible] sociation [illegible] among developmental mechanisms in general (38). A possible explanation on a molecular basis has been suggested (105), but factual information on the subject is very scarce.

E. RESORPTION

Just as the growth of an individual cell [illegible] over [illegible] the [illegible]

intrinsic to the cell groups concerned and on properties of the cellular environments.

The total size of any given central district is the resultant of the *following factors: the proportion of cells in proliferative activity*, the rate of their multiplication, the duration for which they continue to proliferate, the enlargement of cells without further division, the immigration of cells from the vicinity, the loss of cells, and the differentiation of nerve fibers. The classical experiments of Detwiler (20) have shown that the quantitative development of a nerve center is determined both by its own growth potential and by extraneous influences emanating partly from other central regions and partly from the periphery. But it has remained for the painstaking research of Hamburger and his collaborators to reveal the full complexity of these interrelations and to begin to unravel them. For details we refer to the comprehensive article by Hamburger and Levi-Montalcini in this volume.

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in a variety
of character.
constitution-
nterpret the
microscopic similarity of secretion granules in two different glands as implying biochemical identity of either the products or the producing cells. Yet nerve cells, because they all have certain morphological characters in common, are readily assumed to consist of the same protoplasm without specific distinctions relevant to either development or functioning. It is in rectifying this erroneous view that the conference has perhaps made one of its most significant contributions.

The evidence that neuron classes differ in kind and that these differences are instrumental in maintaining both developmental and functional order is based on crucial observations on development, specific reaction to drugs, differential susceptibility to infectious agents, staining reactions, metabolic studies, and functional properties It is especially mentioned in the articles by Schmitt, Bodian, and Sperry. We shall return to it in Section V.

The ontogenetic history of nerve-cell differentiation is complex and still rather obscure. As will be explained in the article of Hamburger and Levi-Montalcini, the neural epithelium is presumably a mosaic of areas the developmental qualities of which have become specifically restricted at very early stages. A good example is the early localization of Mauthner's neuron in amphibians, more fully treated in the article of Stefanelli. While Mauthner's cell presents a uniquely favorable case because of its distinctive size and configuration, the story is likely to be the same for other cell types for which we have as yet found no distinguishing criteria. There certainly exist many more different species of neurons than those at present identifiable as cholinergic, adrenergic, and so forth.

G ELABORATION

Cells emerge from their progressive differentiation not only with

tiation. By bringing cells into novel combinations and interactions, these secondary changes, in turn, set the stage for the next step of differentiation, which will further alter the subsequent distribution of the units, and so on. Thus the pattern of the nervous system is gradu-

ones. In the nervous system, regression has been known to attend the disappearance of body parts during metamorphosis; but, as a component of normal development, resorption of nerve cells has only recently been given prominence, as described in the subsequent article of Hamburger and Levi-Montalcini. Such resorption may be simply the terminal stage of progressive atrophy, which it undoubtedly is after nerve section in later life (see article by Bodian), or it may be due to an active lytic effect of the environment. Whatever the mechanism, it is instructive to realize that, as in so many other phenomena of life, equilibrium is maintained by the proper dosing not merely of a single process but of two opposing principles, one making for production, the other for destruction, operating through different mechanisms.

F. DIFFERENTIATION

Differentiation is frequently understood in too narrow a sense, as the production of *visible* differences among cells. This residue of a pictorially oriented era of microscopic anatomy has given way to a more pertinent concept, according to which differentiation connotes the appearance of *constitutional* differences among cells, presumably always based on biochemical differences but frequently demonstrable only indirectly by the fact that such cells behave differently under otherwise identical conditions (107). Such different behavior may or may not lead to visible distinctions. In this sense, early neuroblasts, spongioblasts, etc., are differentiated relative to one another as well as relative to their own common precursor stages, although the microscope shows no sign of these differences. Differentiation is not a single event but a course of events. Many cells never run the full course of which they are capable but are arrested part way, without "expressing their full potencies," in the usual phrase. On the other hand, many cells are capable, at any one stage of their differentiation, of assuming a variety of appearances, depending on local conditions. Such adaptive modifications of a given cell type have been called "modulations" (89, 107), in contradistinction to the progressive chain of transformations for which the term "differentiation" ought to remain reserved.

For these reasons, the number of truly differentiated cell types in the mature nervous system and the times when they arise cannot be told from morphological observations but must be ascertained for each case by appropriate empirical tests. Just as sheath cells, both embryonic and adult, can modulate into a wide variety of morphological forms, depending on their physicochemical environment (100,

number of central and peripheral functional connections? And so forth.

To many of these questions the conference has given definite, if sometimes provisional, answers or at least plausible suggestions of answers. When brought together from all sides, the scattered bits of information composed a body of knowledge of impressive consistency. In the end a rather sharp picture emerged. It is amusing to reflect on how, only a short time ago, it was deemed perfectly satisfactory to answer all those specific questions by reference to one all-inclusive term—"neurotaxis."

A. ULTRA-STRUCTURE

Marked advances in the study of the ultra-structure and physiology of protoplasm, some on nerve, have constrained our speculations on neuron growth. The article by Schmitt presents an up-to-date account of the ultra-structure of the mature neuron. The article of Flexner establishes the bridge to the embryonic phase. It seems hardly questionable that the capacity to form a nerve process is predicated on the production in the nerve cell of long filamentous protein chains which form the ultra-structural basis of what in the fixed preparation and occasionally in the living (129, 46) appears as a neurofibril. Presumably, the elementary fibrillar units are at first rather mobile and become linked and consolidated into stabler formations only as the axon matures. It would be incorrect to visualize these units as a rigid axis skeleton which, by its crystallization, would protrude the axon. On the contrary, it is rather the streaming forth of the axoplasm from the cell body which orients and aligns the filamentous units contained in it.

B. MECHANISM OF AXON GROWTH

Observations on living, growing nerve fibers, both in transparent tissues of the body and in tissue culture, have produced a rather unified view of their lo[illegible]
of [illegible]
has [illegible]
tip [illegible] part. Elongation results from the deposition of new substance at the tip. This [illegible]
the far-[illegible]
axial st[illegible]
an extr[illegible] in the amoeba, the fluid core of *endoplasm* would gelate upon coming to the surface and thus build up the more consolidated *outer layer* of the axon. Contrary to the amoe-

ally elaborated in an ever increasing complexity of interactions. As a corollary of such differentiation, cells acquire the above-mentioned affinities and disaffinities with regard to other units (105), properties that regulate not only the association between neural and peripheral units (see Sec. II) but perhaps also the relations among neural units.

Genetically, the developmental processes in the nervous system are so timed and dosed that under normal conditions they fit into the developmental pattern of the surrounding body. Whenever this harmony is disrupted, incongruous combinations result. For instance, a normally growing nervous system in a retarded spine and skull will herniate or cave in, with severe functional consequences (133). Evidently there must be all gradations from such crude disharmonies, which are easily spotted, down to the most subtle deviations, detectable only by the closest functional examination. It will be an important task to study the extent to which minor genetic aberrations or nutritional deficiencies during early development leave their marks on the nervous system and hence affect the behavior of the individual (64).

II. THE DEVELOPMENT OF NERVE PROCESSES AND NERVE CONNECTIONS

Ever since the experimental confirmation by Harrison of the outgrowth theory of His, the mechanism of the formation of nerve fibers and the factors controlling their course have been in the foreground of interest. What had at first seemed to be a relatively simple process has presented us with an ever increasing number of facets, each posing a separate problem. For instance: What determines the points at which the axon and later the dendrites will sprout from the cell body? By what forces do they become elongate, and what determines the direction in which the elongation will occur? How do they manage to perforate the limiting membranes of cord and brain, and why do only certain types of fibers emerge? What determines whether they remain simple or become branched and how profuse the branching is to be? Why do they associate with sheath cells and with other nerve fibers to form nerve bundles, and what controls the order, if any, in such associations? Why do they commingle and separate again, as in the formation of plexus, and what determines the final groupings in which they become sheathed by connective tissue? What sets their course in the periphery and what determines where they will end? What controls their density in a given territory and the

2. If nerve fibers advance on a substratum whose fibrous pathways run nearly parallel, all nerve tips are bound to follow the common direction as the only possible one (81, 104, 109, 123).

3. If a nerve fiber advances upon an unoriented network of intersecting fibrils, its tip will become divided at each intersection, with the various branches competing for the inflow of axoplasm. Aside from a factor of inertia favoring maintenance of direction, chance will decide which branch the fiber will follow at each fork. However, the bending of fiber courses observed in the presence of transversal electric fields (50) indicates that electrical asymmetry at the innumerable intersections weights each decision slightly in favor of the cathodal side (109). This does not produce oriented, but merely deflected, nerve growth and operates in combination with, rather than to the exclusion of, contact guidance.

4. If fibrous substrata of different chemical constitutions intersect, a nerve tip exposed to both may choose one kind of pathway to the exclusion of the other. Evidence of such preferential choice between pathways has been obtained *in vitro*, but only on a very elementary scale (104). Much more ... lated to s ... show def ...

... as in larval regeneration (see the articles of Speidel, Piatt, and Stefanelli.

5. Contact guidance is thus a necessary, but not a sufficient, condition for nerve orientation and becomes sufficient only in the special case that all pathways ... tion so as ... orientatic ... actually resolves itself into the production in the nerve environment of just such definite pathway systems. A great variety of experiments has demonstrated that this is achieved by oriented tensions and that such tensions, in turn, are generated by the growth processes of the body itself, through differential expansions and contractions resulting from localized chemical activities as they affect the colloidal substrata (81, 95, 107).

6. Where the structural pathways are indefinit... all ...

... as, for instance, chemical ...ctivity. It seems evident, however, that such chemical guidance, where it occurs, is a matter of contact affinity (105) and not of distance action, as was originally implied in the theory of "chemotropic"

ba, the rear of the cell, being anchored at the central end, cannot, of course, follow the advancing process. As a result, the distance between tip and base increases as more and more substance is drained into the periphery from the nerve cell. The motive force of the elongation is not quite understood. While Lewis ascribes it generally to the contractility of the superficial gel layer, it may become necessary to assume a rhythmic alternation between contractility and relaxation residing in the axon surface and moving the axoplasm forward in the manner of a peristaltic wave. The neuron seems to possess a peculiar pumping mechanism by which material from the cell body can be forced distad all the way to the tip; evidently, this is the same mechanism which, after the fiber has ceased to elongate, continues to pump in axoplasm, which then accrues to the width of the fiber (see Sec. III). At any rate, the elongation of the nerve process is essentially a phenomenon of protoplasmic motion rather than of true growth.

C. ORIENTATION

In nerve elongation the driving mechanism does not of itself determine the direction of the advance. What determines the latter has variously been ascribed to a number of none-too-well-defined tropisms, such as stereotropism, chemotropism, galvanotropism, and hodogenesis. All existing evidence makes it clear, however, that the fiber is guided not by a single factor but by a combination of factors not represented by any one of these simple theoretical categories. The acting principle can best be characterized as "contact guidance" (95), operating through a combination of physical and chemical properties. The following facts seem to be firmly established:

1. Nerve tips cannot extend into a structureless, homogeneous medium but can move only along interfaces (32, 81). Linear interfaces are furnished by the surfaces of all fibrous structures of the surroundings. Even in a planar interface (glass slide, membrane, etc.), there are usually enough inhomogeneities to mark out linear pathways within the plane. Frequently, fibrous exudates coating surfaces appear in the role of pathways (104). The nerve tip is drawn out along such linear threads as if by capillarity. The actual cause of this adhesivity is not known, but it does not seem to reside in simple surface tension and wetting properties, or at least not exclusively so. It exerts a certain pull at the tip of the fiber, thus giving direction to the push of the pumping mechanism discussed before. Because of this dual action, I have termed this a "pull-push mechanism" (103). Guidance by fibrous interfaces is an active principle and not a passive one, such as "least resistance" (95).

The roles of sheath cells and connective-tissue cells in fasciculation and the elaboration of the final architecture of peripheral nerves are practically unexplored. In view of the fact that there is orderly fluid traffic in the spaces between nerve fibers (98, 131), the organization of the nerve as a whole deserves greater attention than if it were a mere package of nerve fibers; hence the development of that organization should receive intensive investigation in its own right and in relation to nerve pathology.

E. BRANCHING AND PLEXUS FORMATION

Terminal branching of nerve fibers can be understood as a statistical consequence of the repeated splitting of fiber tips on a highly intersected pathway system with no specific directional guides (95, 103). Collateral branches, on the other hand, result when the consolidated stem portion of a fiber becomes locally remobilized by some local stimulus (95).

Plexus formation will occur when fibers, arriving from different sources over separate routes, are deflected into a single-track pathway structure (81), such as is furnished, for example, in the dorsal funiculi for the incoming dorsal root fibers, in the various horizontal strata of brain and retina for their radial fiber tracts, in the girdle region for the peripheral nerve fibers, and the like.

F. PERIPHERAL CONNECTIONS

As a rule, nerve fibers of a given kind are found connected with corresponding end-organs As indicated above, end-organs can exert no specific "attraction" on their corresponding neurons but receive their proper neuron consignments already in the right order because of the selective character of the pathway systems leading to the end-organs. However, when fibers arrive in mixed assortments or are forced into the wrong type of periphery by experimental means, the end-organs fail to accept functionally incongruous neurons. Thus cutaneous sensory fibers can be made to grow into muscles, but they will effect no transmissive neuromyal junctions (115, 30). Whether the peripheral matching is any more discriminative than for merely general sensory or motor character is uncertain, since motor fibers connect indiscriminately with any muscle (58) and even intra-central fiber tracts can be brought to innervate muscles adequately (109).

G. SATURATION FACTORS

The number of nerve branches available to a given peripheral area is a function of (a) the number of mature nerve cells in the corre-

nerve conduction. Nerve fibers are definitely guided to their destinations rather than "attracted" by them (124, 109).

The principle of contact guidance, as here presented, can account for most of the known facts of nerve-fiber orientation. It comes closest to the concept of hodogenesis of Dustin (24), or, more correctly, it translates this principle into concrete and analytical terms. Yet fiber orientation is only part of the problem of nerve patterns. There are other contributory features, one of which is "towing" (95). As long as it roams freely, the tip of the nerve fiber determines the course of the fiber. But, once the fiber has attached itself to another unit (muscle fiber, sensory element, or another neuron), its further course becomes subject to passive shifts resulting from the movements and the growth of its terminal organs. Nerves are often dragged over very great distances in the body.

Whether contact guidance, towing, and selective fasciculation (to be described presently) are all that is needed to explain the formation of nerve patterns cannot be decided until certain as yet ill-understood phenomena have been resolved. One such phenomenon is the tendency of nerves innervating limbs with aberrant developmental histories to assume, nevertheless, fairly typical distribution patterns. This was first shown for the nerve patterns in regenerated limbs (128, 57) and then was confirmed for the delayed innervation of originally nerveless limbs (60). Despite minor deviations, the major nerve courses were typical in all these cases, which indicates that the normal chronological succession of events in the ontogeny of the limb cannot be too relevant for the final configuration of the nerve pattern.

D. NERVE FORMATION

Attention has been focused in the past mainly on the orientation of the early pioneering fibers, which grow out singly. In peripheral nerves and central nerve tracts, however, nerve fibers do not remain single but are joined by other fibers that use the pioneers as pathways and thus gradually build up nerve bundles ("fasciculation"). In studying this process experimentally, it was found that fasciculation of significant magnitude occurs only if the nerve finds adequate peripheral terminations (109). Presumably, pioneering fibers which have attained a successful peripheral connection thereby become selectively adhesive for newly outgrowing nerve fibers. Thus nerve cables are established in response to peripheral conditions. This adds another phenomenon to our list of examples of controlling influences exerted by the periphery upon the development of the nerve centers.

The roles of sheath cells and connective-tissue cells in fasciculation and the elaboration of the final architecture of peripheral nerves are practically unexplored. In view of the fact that there is orderly fluid traffic in the spaces between nerve fibers (98, 131), the organization of the nerve as a whole deserves greater attention than if it were a mere package of nerve fibers; hence the development of that organization should receive intensive investigation in its own right and in relation to nerve pathology.

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sponding centers; (*b*) the number of processes that have been drained into the periphery by fasciculation; (*c*) the rate of peripheral branching, which is high in muscle but low in sensory fibers; and perhaps (*d*) the eventual resorption of unconnected fibers. However, from the incoming pool of nerve fibers, only a definite quota is admitted into each peripheral district, the amount being under the direct control of the peripheral tissues. Thus each muscle fiber, as a rule, accepts only a single nerve ending (25), and sensory areas likewise have characteristic saturation densities. Nerve fibers seem to invade a given territory only in proportion to its mass. This has been quantitatively demonstrated for the penetration of regenerating fibers into mature or regenerating limbs of varying sizes (86, 49). In nerve regeneration the peripheral stump itself limits the number of fibers it will carry (48, 113, 114). In sensory territories the self-regulation of innervation density has been directly observed (68). There is no reason to believe that the principle of saturation is in all cases served by the same mechanism. The regional differences of saturation quotas are highly important in their functional implications, since they determine, for instance, the different sensory acuity in different parts of the skin.

III. NEURON GROWTH

Thus far we have dealt only with the numerical development of the nerve centers and the establishment of their topical relations with one another and with peripheral organs. However, in the nervous system the final number of units is established long before the organism has attained its final size, and all further adjustment to the increasing demands is achieved by the continued enlargement of the individual units. Fiber diameter and size of the cell body increase in a certain general proportionality. The final size differential among neurons is based partly on constitutional differences among the nerve cells and partly on influences imposed upon them from their own peripheral organs or from other neurons or, more indirectly, by the degree of functional activity. Studies of neuron metabolism promise deeper insight into the factors regulating neuron growth.

A. *METABOLIC REQUIREMENTS*

The realization that morphological features are merely the outward expression of the behavior of a cell of given biochemical organization in response to its environment is increasingly turning attention from the microscopic signs of differentiation toward the more fundamental biochemical and metabolic criteria. It is only natural that a tissue, even though developed in abnormal surroundings, which has the

morphological aspects of neural tissue has also its biochemical properties, such as the production of cholinesterase (6).

Significant progress has been made in tracing some of the biochemical events attending nerve differentiation, as reported in the articles of Flexner, Bodian, and Hydén. These studies are particularly interesting, in that they demonstrate definite correlations between the development of certain biophysical and biochemical properties and the inception of functional operation in the respective nerve centers.

B. THE GROWTH OF THE NEURON

The growth of the individual neuron has in recent years become a favorite object of investigation. Neurons are uniquely suited for such studies because of their large size and the ease with which the nucleated and nonnucleated portions of the cytoplasm can be separated from each other. The results, obtained by morphological, experimental, and biochemical methods, are in good agreement. They are repeatedly referred to in the articles of Young, Bodian, Hydén, and Flexner. The present state of the field may be summarized as follows:

1. The mass of the neuron, as expressed in the size of the cell body and the caliber of the axon, is not a fixed static character but represents a steady-state equilibrium between continuous growth and concurrent degradation.

2. The site at which the neuron grows is confined to the vicinity of the cell nucleus (perikaryon). Active growth is revealed by increased protein synthesis, enlarged nucleolus and nucleus, and high concentrations of nucleic acids (see Hydén).

3. From the perikaryon the newly produced neuroplasm is conveyed to the axon, where it becomes available both for additional length—during the phase of extension—and for increase in width of the fiber (117). The mechanism through which the proximodistal convection is effected is unknown, but it may be the same as ... discussed in Secti... ing ou ... mecha...

4. While the rate of central synthesis determines the rate at which new axoplasm becomes ... the width of the ch... has acquired a fib... sets an upper limit to the amount of axoplasm that can actually be conveyed to more ... Any marked ... the fiber ...

sponding centers; (*b*) the number of processes that have been drained into the periphery by fasciculation; (*c*) the rate of peripheral branching, which is high in muscle but low in sensory fibers; and perhaps (*d*) the eventual resorption of unconnected fibers. However, from the incoming pool of nerve fibers, only a definite quota is admitted into each peripheral district, the amount being under the direct control of the peripheral tissues. Thus each muscle fiber, as a rule, accepts only a single nerve ending (25), and sensory areas likewise have characteristic saturation densities. Nerve fibers seem to invade a given territory only in proportion to its mass. This has been quantitatively demonstrated for the penetration of regenerating fibers into mature or regenerating limbs of varying sizes (86, 49). In nerve regeneration the peripheral stump itself limits the number of fibers it will carry (48, 113, 114). In sensory territories the self-regulation of innervation density has been directly observed (68). There is no reason to believe that the principle of saturation is in all cases served by the same mechanism. The regional differences of saturation quotas are highly important in their functional implications, since they determine, for instance, the different sensory acuity in different parts of the skin.

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profound influence on our thinking about the integrative action of the nervous system, including the phenomena of learning and memory, which presuppose a certain amount of plasticity of the underlying substratum.

In view of the capacity for continued growth of the neuron, borne out by its faculty for repeated regeneration (see below), it would be wholly inconsistent to assume that any axon has a predetermined length. The fact that intra-central fibers terminate at definite sites must therefore be ascribed to the fact that their free tips have been arrested by junction with local cells rather than by exhaustion of growth potential.

C. TROPHIC EFFECTS

Not only is the metabolic state of neurons regulated by influences from other units, including the terminal organs, but the trophic relation is mutual. Lacking proper innervation, peripheral tissues will atrophy. The time for the deterioration to become apparent varies greatly with the type of tissue and the species, but, as outlined in Speidel's article, it will occur eventually. Whether the atrophy is produced through increased susceptibility of the denervated organs (10) or through the loss of a positive complement normally provided by the nerve fibers has not yet been definitively settled. The trophic influence of innervation on tissue repair and regeneration, while conclusively demonstrated, is also still unexplained.

The trophic control which the nucleated cell body holds over the axonal process has long been deduced from the fact that severed distal fragments of the axis cylinder succumb to degeneration (56). This is easily understood, not only in terms of general physiology but particularly on the basis of what we have now learned about continued protoplasmic replacement from the nuclear center. Pertinent comments on this problem will be found in the article by Gerard.

IV. NERVE REGENERATION

The session on nerve regeneration dealt chiefly with the application of the lessons of primary nerve growth to the processes of secondary regrowth occurring after injury to the mature nervous system. Since ontogenetic and regenerative nerve [illegible]

[illegible]

[illegible] conditions of regeneration in later life, as well as its implications for problems of nerve surgery, justify

tain correspondingly undersized proportions (125). At the same time, excess axoplasm piles up proximally to the constriction because the amount delivered from the cell exceeds the amount that can be carried off through the narrowed passage (117).

5. The amount of myelin produced is a direct function of the caliber of the producing segment of the fiber, so that the wider parts of a fiber have a proportionately thicker sheath than the narrower portions (125).

6. The rate of central synthesis of neuroplasm, which determines the stationary size of the axon, appears to be a constitutional property of the neuron acquired during its differentiation but subject to upward and downward adjustments, depending on a variety of conditions. Lack of adequate peripheral connections entails reduced synthesis, bringing the whole neuron into a lower size class. This effect was first discovered in the reduced calibers of the nerve fibers concerned (124, 116, 62); but, as has later been demonstrated, the primary action is on the cell body (13), in which nucleus and nucleolus are the first to lose size, with the cytoplasm following suit. The proportion of neurons in which atrophy after peripheral disconnection comes to rest at a lower size level seems to vary with the kind of neuron and the species. A certain proportion shrinks to the point of nonviability and is resorbed. Perhaps the difference between peripheral and central neurons in the ability to regenerate axons, discussed in Section IVF, is an expression of the different degrees of regression suffered by different cell types upon the amputation of their processes. These problems are ably dealt with in the work of Bodian.

7. Conversely, when a neuron is overloaded peripherally by excessive branching, it undergoes a certain degree of hypertrophy (see the article of Young). A similar increase is also obtained as a result of intensified physiological activity (see Hydén's article).

The size fluctuations of the central cell body and its peripheral fiber in response to varying demands for growth and physiological activity are of far-reaching importance for the general understanding of neural functions (see Young's article). Let it be remembered, for instance, that with the size of a nerve fiber are correlated such physiological properties as threshold, sensitivity, and conduction velocity, and that the size of the cell surface, in combination with some saturation factor as discussed in Section IIG, will determine the number of synaptic endings on that unit. Above all, the realization that the neuron is a system in a constant state of flux must have a

profound influence on our thinking about the integrative action of the nervous system, including the phenomena of learning and memory, which presuppose a certain amount of plasticity of the underlying substratum.

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a treatment in its own right. In this treatment the conference dwelt as much on the basic similarities to ontogeny as on the distinguishing features, which in some respects, particularly that of numerical control and of specificity of pathways, are quite marked.

A. MECHANISM OF PERIPHERAL NERVE REGENERATION

The cut end of a proximal nerve-fiber stump establishes a new growing point which advances by the same type of amoeboid motion that characterized the first outgrowth. There seem to be the same mechanisms at work, and even the rate of advance is of the same order as in first development, namely, at best, several millimeters per day in warm-blooded animals. As in first development, fibrous pathways of the surroundings act as guides to the advancing tips, and the abundant branching and extensive straying of young sprouts near the wound is definitely attributable to the irregular and confused arrangement of the pathway system in the fibrous scar that forms over the nerve end. As in first development, it has been possible, by the application of longitudinal stresses, to orient the fibrous pathway system along the nerve axis, thus giving the outgrowing sprouts a straight trellis that guides them over the gap to the distal stump (97, 101, 103, 123). The orientation of the fibrous substratum under the influence of stress is reinforced by proteolytic resorption of all fibrous cross-links that do not lie in the direction of major stress (123).

Since such fibrous pathways serve as guides for sheath cells as well as for nerve fibers, the association between these two elements remains close. In the distal stump the nerve fibers likewise find ample sheath cell tracks to associate with; and there seems little doubt that these two tissue elements are predisposed by their mutual affinities for a true symbiotic relationship (1). This matter is treated from the classical histological point of view in the article by Boeke. If the Schwann cell contributes any nutriment for the formation of the axon, it is obvious from Section IIIB that this could not be incorporated at the place of intake but would have to be moved centrad for assimilation. The main function of the Schwann cell seems to be to coat fibrous interfaces with its substance, which is peculiarly suited for the application of nerve fibers, and, moreover, to provide the stimulus and perhaps some complements needed for the formation of the myelin sheath.

B. REGENERATION RATE

Nerve fibers can regenerate repeatedly with undiminished vigor and unreduced rates (23). Since the advance of the tip is physical

motion rather than true growth, the so-called "regeneration rate" is presumably determined chiefly by the physical properties of the system, such as contractility, viscosity, elasticity, etc. It is interesting to note that the rate of advance (31) is of the same order as that which has been calculated for the rate of protoplasmic replacement in the continuously growing neurons (117). Emphasis on the physical properties does not mean, however, that these processes could go on in the absence of the necessary metabolic, i.e., biochemical, resources.

Maximum rates of advance of *ca.* 4 mm. or more per day are obtained when the free tip can proceed on a straightaway, either along a column of Schwann cells (29) or in a well-oriented fibrous matrix with large liquid interstices (123). Wherever these optimum conditions are not realized, the regeneration rate is reduced, evidently in proportion to the frequency of temporary arrests, which, in turn, is a function of the intersectedness of the fibrous reticulum along which the fiber tip must proceed (104). An apparent stimulation of the rate of nerve-fiber regeneration after systemic administration of certain nerve extracts, reported in the literature (53), is clearly to be explained as an effect of the agent on the consistency of the scar, reducing resistance to nerve-fi[illegible]
fiber "growth." Since w[illegible]
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regeneration by applying so-called "growth stimulants" miss the mark, and actually every single one made in the past has failed.

C. SELECTIVITY

As in first development, the principle of contact guidance has been found fully valid for nerve regeneration, essentially as defining the possible routes rather than the actual courses of a given fiber. Only if the substratum is strictly oriented (see above, Sec. IVA) will this provision also define the actual course of the nerve. Otherwise, the problem of additional factors selecting one pathway to the exclusion of others raises itself. In this, however, regeneration of mature nerves is significantly different from first development. Thus far, no evidence of specificity of pathways has been found in regeneration. Sensory nerve fibers travel as readily and as rapidly along former motor nerve paths as along their own (115, 30), and motor fibers grow with equal ease through sensory stumps (114). Likewise, a denervated muscle is as ready to accept innervation from a foreign nerve as from its own former nerve (118, 25). Since some degree of selectivity has been re-

ported for the regeneration of nerves in larvae (73; see also Speidel's article below), we are evidently dealing here with a property that is only gradually lost in the course of development and maturation.

Contrary to the lack of selectivity during regenerative outgrowth, peripheral connections are established only between nerve fibers and end-organs that match. Thus sensory fibers will pervade a muscle but will fail to establish transmissive connections (115, 30). The fact that the presence of such regenerated fibers cannot be demonstrated by functional tests formerly led to the erroneous interpretation that fibers fail to regenerate into nerve stumps of a different kind (18).

D. NERVE GRAFTS

Nerve grafts are intended to reduce the gap between separated nerve stumps which regenerating fibers must span before entering the protective and guiding channels of the distal Schwann tubes. In animal experimentation such grafts often serve the intended function admirably (61), even after devitalization (122, 99) that does not transform them into foreign bodies. However, the usefulness of grafts in peripheral nerve surgery in man is still seriously questioned (65).

E. CLINICAL ASPECTS

The emergency of the second World War brought a new upsurge in interest and research to improve methods of surgical repair of injured nerves. While the urgency of the situation dictated for the most part an empirical approach, attempts to broaden and strengthen the factual and theoretical foundation on which all nerve repair rests have not been neglected. By applying the knowledge of the mechanisms of nerve regeneration to practical problems, some distinct advances have been made (66, 101, 103, 126, 51, 4, 71). However, the technics thus developed on laboratory animals have not always proved applicable to clinical practice, and, even where application was feasible, the results in man did not equal those obtained in the laboratory. This is due to the fact that, despite the fundamental identity of the processes of nerve regeneration in man and other mammals, differences of size and degree often prove of crucial importance. Some comments on the problem of nerve regeneration from the viewpoint of a clinical investigator are reported in the article by Sunderland.

Even when morphologically adequate peripheral nerve regeneration can be obtained in satisfactory volume, the functional result can never measure up to the normal condition as it existed prior to the injury. This is due to the fact that, because of the lack of specificity in nerve regeneration, a majority of the fibers enter into more or less

foreign paths. Channels leading to incongruous endings are thus wasted on functionally irrelevant regeneration, while channels leading to functionally related, but still aberrant, destinations will cause a corresponding confusion in motor co-ordination. Some of this can be corrected by functional adjustments in the central patterns of co-ordination; but the range within which such reorganization is possible is definitely limited and much more modest than former sweeping generalization concerning the plasticity and regulability of central nervous functions would have made one believe (111, 70).

On the other hand, further improvements in the technics of nerve repair can be safely predicted if systematic research is continued. In this pursuit it would seem profitable to go beyond the mere reiteration of the fact that there are certain crucial differences in nerve regeneration between man and experimental animals and to find out just what those differences consist of and how to make proper allowances for them in extrapolating from laboratory mammals to man.

F. CENTRAL REGENERATION

Peripheral nerve regeneration is essentially a phenomenon of cellular physiology, inasmuch as it concerns the self-repair of a mutilated cell, the neuron. In contrast to this highly developed faculty, the nervous system has no regenerative capacity on the tissue level, that is, it cannot replace whole neurons that have been lost. There is some capacity for central regeneration during embryonic stages and in later life, at least in the lower vertebrates such as fishes and amphibians (see the article on central regeneration by Stefanelli), but in mature higher forms any loss of neurons is *permanent*. Reparative processes in the centers remain confined to proliferation of scar tissue, largely composed of glia.

As for the capacity of central neurons to regenerate lost cell processes, it seems rather definite that such ability is less widespread and relatively minor in degree as compared to the peripheral system. A discussion of this point will be found in the articles of Gerard and Hooker summing up succinctly the present status of the problem. More concerted research along these lines may bring more conclusive solutions, which would be eminently important in order to assess the possibility, however remote, of repairing central lesions in man. If the poor regenerative display in the centers is due to unfavorable conditions in the neuronal environment, there may be hope for a solution. If, on the other hand, as indicated in Section IIIB6, the incapacity is based on constitutional properties of the central neurons

themselves, then there would indeed be much less reason for optimism.

V. SPECIFICITY AND SELECTIVITY

One of the serious defects of most modern neurological and neurophysiological theories is their patent neglect of the qualitative diversity of the neural elements. Psychology is forced to postulate specific differences in neural activities, and experimental biology can demonstrate them *ad oculos*. Yet most physiological concepts of central nervous activity proceed from the assumption that all neurons are essentially alike in character, that is, are all constituted of essentially the same kind of protoplasm, and that the only parameters relevant to central nervous activity are quantitative variations of the metabolic state of this protoplasm, of the electric properties of its surface, and of the geometric configuration of the network. This thesis of the uniformity of the neural network has found its most explicit expression in the current comparisons of the brain to an electronic calculating machine (132).

In this situation it becomes doubly important to stress the evidence that has accumulated in favor of the inner qualitative diversity of the nervous system. It was interesting to note that the conference repeatedly took occasion to emphasize this fact of specific differentials among neurons, to which direct reference is made in the articles by Schmitt, Bodian, Speidel, Young, and particularly Sperry.

The problem of specific differences among neurons has, of course, several aspects. The first question is whether such intrinsic differences exist; the second, whether they are demonstrable; the third, whether they play a role only in the developmental process; the fourth, whether they are instrumental in establishing functional order; and the fifth, whether they can be described in terms of known physical and chemical properties.

A. CONSTITUTIONAL SPECIFICITIES

The evidence for the existence of constitutional differences among neurons beyond the crude subdivisions into cholinergic and adrenergic neurons has already been referred to above, in Section I. The embryological evidence alone is quite conclusive. In addition, there are the differential reactions to drugs, stains, viruses, and other agents already mentioned. It is interesting to note that many students of the physiology of the nervous system are still so strongly dominated by the spirit of a strictly morphological era, in which only visible images were rated as valid criteria, that they are willing to

concede biochemical specificity to a nerve cell in the pre-optic nucleus of a lower vertebrate, in which certain secretion granules can be seen under the microscope, but would be reluctant to admit the existence of similar specificities in other neurons with no directly visible distinctions, even though behavioral tests of those neurons contain conclusive proof of their distinctiveness in development and response.

B. SELECTIVITY IN NERVE GROWTH

The case for selectivity with regard to pathways and end-organs during the primary outgrowth of nerves has already been presented in Section II There is no need to restate the case here, but it is necessary to point out the full meaning of its acceptance. It should be borne in mind that acknowledging the selectivity of a given neuron type or a given pathway or a given end-organ implies nothing less than the following: that different neuron types are fundamentally and critically different in their composition; that a similar critical diversity exists among pathways and terminal organs; and that the neuron must possess means to identify or recognize, as it were, the appropriately matching specificity of its predestined pathway or terminal These are logical inferences which are cogent, irrespective of whether or not we find any explanation of the underlying differentials in the realm of known physical and chemical phenomena. In an effort to show at least the *feasibility of such* an explanation, I have proposed a stereochemical model of the type of affinities involved (95, 105), which, pending more precise information, gives us at least a formal picture of selectivity. Its pertinence remains to be proved. At any rate, let us remember that the failure of a sensory neuron to connect with a muscle fiber lies in subtle discordances on the molecular level which the microscopic picture is too crude to reveal. This being the case, one should not *expect that* looking through the microscope at a synaptic ending in the central nervous system would reveal with any greater degree of certainty whether or not the particular contact is transmissive.

C. MODULATION

Perhaps the most astonishing, though incontrovertible, evidence of specificity not only in nerve *growth* but in the establishment of functional order in the nervous system has come from the so-called "resonance" phenomena, through which particul[illegible]
them[illegible]
cen[illegible]
co[illegible]

review (108). It is dealt with rather concisely in the article by Sperry in this book.

In a long series of experiments it could be shown that each muscle has a specific biochemical differential, that it projects this differential into the motor nerve fibers that come to innervate it and thus tunes ("modulates") the motor ganglion cells to a specificity appropriate for the particular muscle. The central nervous system, in turn, employs in its co-ordination corresponding sets of specificities, so that central activity destined for, let us say, a gastrocnemius muscle is selectively picked up by the gastrocnemius-specific ganglion cells to the exclusion of all others ("myotypic function" [94]). The significant point to remember is that these ganglion cells have received their specificity by a retrograde influence ("modulation") from the muscle itself. This is proved by the fact that, upon reconnection with another muscle at a sufficiently early period of life, their specificity and central response relations will change in accordance with the new termination (75, 78, 85).

The same type of specific relationship, based not on stereotyped connection patterns but on secondary modulation, has also been demonstrated within the proprioceptive field. It was discovered that the central nervous system identifies, as it were, an excitation coming from a particular muscle according to the name of the muscle rather than according to its place in the system or according to the mechanical, physiological, or biological effects of its contractions (127). A further example of specific modulation was found in the establishment of the cornea reflex (96). The most remarkable demonstration of subtle specificities in the sensory field has certainly been furnished by the experiments of Sperry on the visual sense. These and other related experiments are reported in greater detail in his article.

It would seem idle to speculate on how far the continuation of this line of research will lead us. That these resonance relations are at present wholly outside the sphere of physiological thinking is evidenced by the simple fact that, obviously, no one could have predicted the results from existing theories. It is perhaps equally idle to speculate about the reason why these phenomena, which were first described nearly thirty years ago, have remained essentially unassimilated by the body of neurological concepts. It would seem that additional research, employing a combination of the classical technics of nerve physiology with the experimental technics of transplantation used in experimental embryology and morphology, could go far in removing the existing inconsistencies.

VI. DEVELOPMENT OF BEHAVIOR

It is a healthy sign that the sharp separation once advocated between a purely phenomenological study of behavior, on the one hand, and the physiological study of its possible neurological foundations, on the other, has not been generally adopted. The pursuit of any one scientific field under an injunction against trespassing into another is neither rational nor productive, especially if both have common objects It simply is not true that nothing can be learned about the "organism as a whole" by studying its constituent parts and their interrelations. On the other hand, it would, of course, be equally erroneous to assume that mere preoccupation with the elements will tell the full story of their collective behavior (76). In the light of developments, it would seem unwarranted to subscribe to either a purely holistic or a purely elementarian theory of neural functions and behavior to the exclusion of the other, or to pursue studies on behavior alone or on its neurological foundations alone without the benefits that each field can derive from the advances of the other. Regardless of the pertinence of his detailed propositions, it certainly has been the historical merit of Coghill (16, 34) to have made a strong case for the conjoint attack on the problems of behavior and against the separatist trends of technical disciplines.

The realization that much can be learned about behavior by the study of its development is of relatively recent date. But, as frequently happens in the history of science, the formation of theory outraced the acquisition of factual knowledge, and soon students of the development of behavior were found to be rallied around two opposite doctrines, one stressing the primacy of the holistic, the other the elementarian, viewpoint. Each centered its arguments on certain objects, observations, and technics different from those of the other, and evidently each party felt justified in considering its particular niche as a fair sample of the behavioral universe. Thus what in sober evaluation would have become a fruitful stimulus to further clarification of the issues assumed the dogmatic aspect of an irreconcilable antithesis. Again, as often happens in the course of scientific history, the conflict is turning out to be a matter of one-sided viewpoints and undue generalizations rather than of facts. The articles of Windle and Hooker give a brief account of some of the recent history, and the article of Barron illustrates well the healthy tendency to escape from the dilemma and reconcile contradictions based partly on interpretation but partly also on a real diversity inherent in the various objects. It was highly encouraging to the conference to see how the

review (108). It is dealt with rather concisely in the article by Sperry in this book.

In a long series of experiments it could be shown that each muscle has a specific biochemical differential, that it projects this differential into the motor nerve fibers that come to innervate it and thus tunes ("modulates") the motor ganglion cells to a specificity appropriate for the particular muscle. The central nervous system, in turn, employs in its co-ordination corresponding sets of specificities, so that central activity destined for, let us say, a gastrocnemius muscle is selectively picked up by the gastrocnemius-specific ganglion cells to the exclusion of all others ("myotypic function" [94]). The significant point to remember is that these ganglion cells have received their specificity by a retrograde influence ("modulation") from the muscle itself. This is proved by the fact that, upon reconnection with another muscle *at a sufficiently early period of life, their specificity and* central response relations will change in accordance with the new termination (75, 78, 85).

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behavioral development does not apply equally to the terminal phases, in which the inherent developmental patterns are polished and perfected by actual practice and adjustment.

The phenomenological study of the development of behavior has revealed that, like all development, it follows a trend from the general to the specific and from more widespread involvement of elements to more restricted and differential activation. Coghill's principle of "individuation" from a background of mass reaction is based on this realization. It still holds true as designating a trend of events, even if the initial performance under consideration has never been a total activity of the whole body. In some cases and for some functions the primordial activity undoubtedly involves all the neural apparatuses capable of functioning at the same time (see subsequent articles by Hooker and Barron), while in other cases and for some other functions it seems equally clear that activity is territorially localized from the beginning (see the article by Windle).

To give a clear-cut example of the latter type, we need only refer to the appearance of the lid-closure reflex in amphibians studied extensively in our laboratory (41, 43). This reflex appears only at metamorphosis after having been completely absent in the otherwise fully functional larva. Both the sensory and the motor innervations required for the reflex are present in the larva and are individually capable of functioning, but the necessary central relations are not established until a certain state of maturation is reached. As soon as these relations are established, the reflex makes its appearance as a strictly localized and circumscribed act, which has never been part of a "total pattern" of central functions. Evidently, individuation from mass action does not apply to this type of response, but this, in turn, does not invalidate the principle for other performances. The sober- [illegible] component of behavioral development in its own right

C THE NEURAL BASIS OF INDIVIDUATION

The development of behavior shows clearly two phases—an early expansive and a later restrictive one (12). During the [illegible] phase [illegible]

This [illegible]

the [illegible] central nerve connections and pathways (16). In a sense this correlation is obvious, as it merely expresses the fact that where there is no neural pathway there can be no neural

spirit of exploration overcomes doctrinary ties and how research on the development of behavior and of its neural basis is proceeding with renewed vigor.

A. BEHAVIOR AND CHEMISTRY

Valuable facts have come to light in the study of chemical systems known to be indispensable for functional activity in the mature nervous system. The appearance of the cholinesterase system, as well as its quantitative development, is closely correlated in time with the appearance and maturation of the functions of the central nervous system (118). Similar and even more subtle correlations between the appearance of chemical and functional properties have been summarized in the subsequent article of Flexner. Perhaps many of these facts prove no more than that the nervous system, in order to operate properly, must be in possession of all the physical and chemical properties known to be requisite for such functioning. But some future refinements of tests may lead to a more differential diagnosis by which the *simultaneous appearance of a particular compound and of* a particular functional complex would reveal specific causal links that could not be isolated from the fully developed nervous system in which all the chemicals and all the functions coexist. The study of development may thus be used on a larger scale in the same analytical sense as the study of deteriorating functions in mental patients has been used to identify certain biochemical correlates of central activity (35).

B. PHENOMENOLOGY OF THE DEVELOPMENT OF BEHAVIOR

The phenomenology of behavioral development is actually an old discipline. It started with the recognition of the fact that behavior does have a stepwise ontogenetic history, and it went on to describe the steps involved. Only in the second instance did it proceed to test the significance of the steps as instruments or causal links in the development of the whole sequence. However, ever since the demonstration that embryos raised in narcosis would develop behavioral patterns of normal organization (11, 52), even though the overt expression of the whole series of precursor steps had been suppressed, it has been clear that the behavioral steps are merely external manifestations of underlying intrinsic developments rather than practice steps. The complex performances of later stages cannot possibly be founded upon the tested success of their simpler precursors, since they seem none the worse for the omission of the intermediate functional tests due to narcosis. Again, undue generalizations must be avoided, and what is said here for the early and fundamental steps of

areas develop without major impairment, and limbs lacking sensory innervation from the beginning function co-ordinately without sensory control having ever had a chance to play a constructive part in the development of the motor patterns (93, 135, 22).

E. THE ORIGIN OF CENTRAL CO-ORDINATION

Since neither learning nor patterns of sensory stimuli have any part in the development of orderly central functions, we must look to the autonomous processes of central development itself as the source of co-ordination. Experimental evidence indicates that the substratum of co-ordination is not spread diffusely over the nervous system but is restricted to sectors related to the corresponding organs; for limb movements, for instance, it resides in the limb segments of the cord. Detwiler was the first to show that a limb innervated from trunk segments cannot function properly (20). Limbs innervated from cranial nerves are similarly incapacitated (59). On the other hand, trunk segments incorporated in the limb level of the cord at an early embryonic stage will acquire the functional organization requisite for limb co-ordination (20).

We must recognize, therefore, the existence of specific regional differences in the establishment of co-ordination patterns, but the nature of those differences has not yet been disclosed. The only fact that has been shown conclusively by experimentation is that the central nervous system develops a finite repertory of behavioral performances which are pre-functional in origin and ready to be exhibited as soon as a proper effector apparatus becomes available (92, 94).

F. SPONTANEITY

A clear distinction must be made between the generation of a central discharge and the pattern of its distribution (co-ordination). Contrary to a widespread belief, a central discharge does ...
for ...
cen ...
hav ... referred to underlying fluctuations in the metabolic and electric state of the neurons (26). The discovery that any isolated and deranged fragment of medulla or spinal cord will permanently exhibit trains of spontaneous rhythmic discharges (90, 91, 109) suggests that such activity is a basic property of pools of neurons rather than a specialty of certain centers only (92).

G. HORMONE EFFECTS

One of the most interesting ...
genetic neurology is ...

function. Reactions during this early phase are remarkably stereotyped, indicating absence of discriminative response mechanisms. However, the more the nervous system approaches structural completion, the more prominent becomes its ability to activate restricted portions and patterns of the existing network independently in selected and co-ordinated combinations. It is this restrictive "individuation" for which the proper neural correlate is still to be revealed.

Coghill and others believed to have discovered a general neural model of "individuation" in the development of limb innervation. Amphibian limbs were described as being moved at first only in association with trunk movements, which was explained by the fact that their early innervation consists of collaterals from the motor neurons of trunk muscles. Later "dissociation" from the trunk appeared linked to the development of a secondary separate fiber system from the limb segments of the cord (137). Closer study (72), however, suggests that the so-called "primary" associated limb movements are, in reality, passive movements effected through the trunk muscles of the shoulder, while the intrinsic true limb movements do not appear until after the limb muscles have received the independent set of segmental neurons which was called "secondary" but which, as far as these muscles are concerned, is really primary. Hence the intrinsic limb function arises as a separate and individualized activity from the very first rather than as an "individuated" offshoot of an earlier mass response. There being no individuation, the attending neural changes cannot possibly serve as a model to explain individuation, and the search for the neural correlate of individuation must continue. The progressive refinement of the control of movements within the limb, which constitutes "individuation" on a smaller scale, can be accounted for, at least in part, by the progressive muscle-specific modulation of the limb neurons, establishing more discriminatory central relations (see Sec. VC and the article by Barron.

D. CENTRAL ORIGIN OF CO-ORDINATION

Many modern concepts of neurophysiology attempt to derive the properties of the output of the nervous system directly from the pattern of the sensory input. Such a concept is clearly contradicted by the studies on development. The fact that the appearance of motor performance antedates sensory control has often been stressed (16). Even more striking is the evidence of animals in which the development of the sensory nervous system had been experimentally suppressed. The basic patterns of motor co-ordination in such anesthetic

resulting in the determination to combine their efforts wherever possible in a conjoint attack on the vast unsolved problems of genetic neurology.

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havior that accompanies the transition from the larval to the adult stage in amphibian metamorphosis. During this phase many new structures and functions arise, while old ones disappear, and the nervous system undergoes a thorough remodeling so as to fit the reorganized system. It has been shown that the thyroid hormone, which actuates the bodily transformations, has also a direct and local effect on the nerve centers that are to be transformed (42). This may be a pertinent model of the hormone dependency of neural and behavioral changes in other instances, such as the activation of sex behavior at the time of sexual maturation (5). It appears that hormone action has no constructive or pattern-determining influence on the functional performance but operates merely by releasing certain patterned responses the distribution of which is determined by differential susceptibilities acquired during previous phases of differentiation.

II. CONCLUSION

The points chosen here for special comment were to be merely illustrations of how the analytical technics of experimental embryology and experimental morphology can be profitably applied to the study of the development of behavior, resulting in clarification of our concepts of behavior and its neural basis in general. Here the achievements of the conference acquire higher meaning. Just as the application of nerve regeneration to the problems of surgical nerve repair in the fourth session, so here, again, the demonstrated bearing of biological research on problems with which human neurology, psychology, and psychiatry are still grappling presents a strong case for the inseparability of fundamental research and human welfare. While scientific workers are more and more constrained into narrower and narrower confines in which to pursue their specialties, science as a whole cannot develop as a healthy and proportionate organism unless specialists will leave their burrows on periodic occasions and meet on common ground, take stock of their common inventory, sort out and interrelate their discoveries, survey the perspective of the whole, and develop the habit of keeping one another's problems and concepts in view while working toward a common goal, albeit through separate channels. It would seem that this conference has been eminently successful in achieving just this informal integration of viewpoints. It has brought clarification of old concepts as well as the formulation of promising future research, but, above all, it has given all participants a lively awareness of the interdependence of their various specialties,

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would undoubtedly be preoccupied with the mechanism of impulse propagation along the fiber or across synapses, the other processes would be equally absorbing to various types of biologists. One colleague, a distinguished embryologist, considers impulse propagation among the less interesting of nerve phenomena, a secondary rather than a primary process.

To what extent can a definitive relationship between structure and function in nerve be demonstrated? During the last century many attempts have been made to deal with this problem at the histological level, but the net results are unimpressive. Particularly unrewarding have been the studies of the morphological basis of impulse propagation. Since the physiological process occurs at the molecular or atomic level, experimental technics must be capable of dealing effectively with changes occurring at that level. The morphologist has still a long way to go before his tools are sufficiently sharp for this purpose. However, some progress has been made at the colloidal level by polarized-light and x-ray diffraction analysis of neuron ultrastructure, and electron microscopy now promises to extend our knowledge considerably further.

It will be the purpose of this paper to present a brief critique of some of the results obtained thus far and to mention certain very recent work on this subject done in our own laboratory. Since most of this work has been done on the peripheral nerve fiber, the discussion will be limited to this portion of the neuron. The application of similar methods, particularly that of electron microscopy, to the cell body, the synapse, and the terminations of the fiber in the end-organ should prove fruitful fields for further investigation.

All nerve fibers have the common property of propagating impulses and of manifesting certain trophic processes of maintenance and repair. Although various types of nerve fibers show great diversity of structure, chemical composition, metabolism, and physiological properties, it will be useful to assume that a common structural and chemical pattern is present in all types. The differences, which may appear to be great from one type of fiber to another, may be regarded as variations on the main theme rather than as distinctly different idioms. It will be our present purpose to focus attention upon the common factors of the nerve fiber from the structural and chemical aspects.

Experience with other physiological problems suggests that this may prove a fruitful method of approach. Consider the case of muscle. Since the earliest work on muscle, it has been assumed that

THE COLLOIDAL ORGANIZATION OF THE NERVE FIBER[1]

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THE neuron is an extremely complex system capable, under certain conditions, of manifesting a wide variety of physiological processes. If we were able to view the neuron through atomic spectacles, so that we could discern not only the colloidal structure but also the details of molecular and atomic configurations and the chemical reactions which occur, we should probably be able to recognize at once the process of impulse propagation because of its time relations. However, it is probable that most of the observed structures would have little obvious relation to impulse propagation but would be identified as associated with one or another of the manifold functions of the neuron. One would expect to observe the molecular apparatus by which processes such as the following are made possible: repair and regulation after injury; the maintenance of so improbable a structure as a cylindrical cell outgrowth which may be hundreds or thousands of times longer than the diameter of the cell (possibly involving a continuous movement of axoplasm from the cell body peripherally [1, 2]); the synthesis of proteins and of nucleoprotein particulates and their breakdown, as in chromatolysis; the liberation of ions and of humoral agents, such as acetylcholine, at the end-organs; also certain poorly understood processes, such as those which occur in the cell body very soon after section or injury to the fiber at a point far removed from the cell or after other types of severance of the neuron from its peripheral field. Quite possibly a portion of the molecular apparatus might represent a residuum of the initial process of growth and differentiation by which the fiber grew from the cell body to the specific end-organ during embryogenesis—a truly remarkable phenomenon when viewed in all its aspects. Although most physiologists, looking through the atomic spectacles,

THE MYELIN SHEATH

From the polarized-light and x-ray diffraction studies of vertebrate nerves, certain significant features of the structure of the myelin sheath have become apparent (4, 5). The lipid molecules are oriented with paraffin chains extended radially, constituting *smectic mesomorphic* layers of mixed lipids. The organization *within* the planes of the lipid double layers is essentially that of a liquid. The repeating period of the myelin sheath in the radial direction is remarkably constant in the nerves of the various vertebrates thus far studied: about 171 A in amphibians and reptiles and about 186 A in mammals. Quite possibly the longer period in mammalian nerves may be due to the presence of longer paraffin chains in the lipid molecules or to a different proportion of the lipid types. The evidence seems clearly to require that, besides lipids and water, proteins enter into the radial repeating period. This is presumably the protein classically called "neurokeratin." Unfortunately, definite knowledge about this protein is very scanty. The sulfur-containing pseudo-keratin isolated from nerve has not been definitely localized in the myelin sheath, and, indeed, Block (6), who made the analyses, considered that it may more probably be an axonic constituent. Folch and his collaborators (7, 8) have isolated and characterized several liponucleoproteins and proteolipids from brain. Whether any of these represents the protein of the myelin sheath cannot yet be stated.

In Figure 1 is shown a schema of the structure of the myelin sheath as suggested by Schmitt, Bear, and Palmer (5). From a study of purified lipids in single components and in mixtures, the probable thickness of the double layers of mixed lipids in the myelin sheath has been estimated. From the decrease in the repeating period when nerves are dried, it was estimated that the thickness of the water layers is about 25 A This leaves about 25–30 A for the thickness of the protein layer The behavior on drying suggests that certain of the lipid molecules may be firmly bonded to the protein, possibly [illegible] lipid-protein [illegible] cate [illegible] this may become poss[illegible] the protein, or lipid-protein complex, of the myelin sheath is isolated.

The model of sheath structure in the radial direction is meant merely as a guide for further study; the diffraction data do not permit a more definitive description at the present time. The radial repeating period is fairly well established. In a myelin sheath having a

despite great variations in morphological and physiological properties manifested by different types of muscles, a common system of fibrous proteins is involved in all contractile tissues. With the advent in recent years of more detailed knowledge of muscle structure and with better characterization of the protein components, it seems probable that the *actomyosin complex in its relation to adenosine* triphosphate, adenosine triphosphatase, and inorganic ions may be common to all contractile tissues. What other substances form integral parts of the system is still a matter for debate and research. However, these partial systems, capable of manifesting certain forms of mechanical alteration, have greatly advanced our understanding of the contractile mechanism.

To what extent have such common factors been defined in the *peripheral nerve fiber? From the physiological viewpoint*, one may assume that all nerve fibers possess a metastable, paucimolecular membrane or film of unknown composition, presumably located near the surface of the fiber, and an axoplasm capable, through reactions of intermediary metabolism, of furnishing the energy necessary for maintenenace of specific structure and for propagation of impulses. The morphologist will probably insist that all nerve fibers possess neurofibrils, which, after appropriate histological preparation, may be demonstrated to course through the axon of the fiber, and an axon sheath, which may vary in thickness and lipid content from the well-developed myelin sheath of the large A fibers to the thin, "unmyelinated" type.

From the chemical viewpoint, the least common denominators of nerve are even less clearly defined. Certain lipids (phosphatids, cerebrosides, and steroids) are present, but these are fairly common in most tissues; with few exceptions (3) they are not unique to nerve. The proteins, which are the most characteristic tissue components, are at present poorly characterized in nerve, as will be discussed below.

Perhaps the common factors of nerve-fiber organization are the surface membrane and the axon (axis cylinder). However, since the axon sheath varies from a well-developed myelin sheath, through all transitions of decreasing lipid content, to a surface film and since more detailed information exists about the ultra-structure of the myelin sheath than for any other nerve structure, it will be included in the discussion. The individual items, then, are the myelin sheath, the surface film, and the axon.

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The model of sheath structure in the radial direction is meant merely as a guide for further study; the diffraction data do not permit a more definitive description at the present time. The radial repeating period is fairly well established. In a myelin sheath having a

thickness of 2 μ there would be over a hundred of the repeating periods if continuously packed in the sheath. However, considerations of the water content of the myelin suggest that the sheath may contain slabs of such organized myelin separated by aqueous channels.

No detailed electron-microscope studies of the myelin sheath have, at this writing, been published. Layered fragments, possibly deriving from the myelin sheath, have been observed in our laboratory by Sjöstrand (9), as well as by De Robertis and the author, in fragmented preparations of fixed nerves. Similar structures have been observed by Fernandez-Moran (10). From the x-ray data it might be expected that the layered structure would be resolvable with the

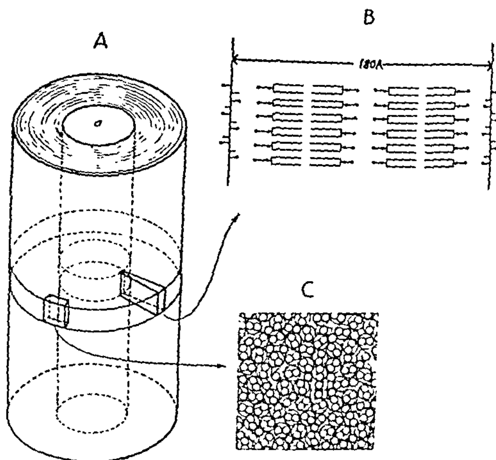


FIG. 1.—Diagrammatic representation of the myelin sheath structure as deduced by polarized light and x-ray diffraction studies. *A* shows the lamellar structure of the sheath. [illegible] in the sheath. No attempt [illegible] ch form mixed lipid phases. [illegible] shown as globular particles, since at present there is no evidence concerning the nature of this protein. *C* shows a plane through a lipid layer, i.e., tangentially in the sheath. Small circles indicate individual hydrocarbon chains, large circles indicate the domains of individual lipid molecules.

electron microscope, since the repeating period is 170–90 A (or possibly about half this figure, if the individual double layers are resolved). Fernandez-Moran (11) has observed a banded appearance in electron micrographs of thin sections of mammalian myelinated nerve fibers which he believes represents an aspect of the concentric layered structure of the myelin sheath demonstrated by x-ray studies. On less convincing grounds Fernandez-Moran believes that a filamentous network observed at the periphery of the axon in transverse sections represents the axolemma, postulated in the histological literature to occur between the axon and the myelin sheath. Thus far no electron-microscope observations have been made of the detailed structure of the nodes of Ranvier, Schmidt-Lantermann incisures, Golgi funnels, and the other constituents of the *myelin sheath*.

As shown first in the case of lobster and *Limulus* nerves (12) and subsequently for the giant fiber of the squid (13), invertebrate axons [illegible] The concentration of lipids is so small as to require special methods for demonstration. Thus far no x-ray long spacings corresponding to the lipid-protein repeating layers have been observed in invertebrate nerves. It is therefore impossible to say anything about the detailed structure of the thin axon sheaths of these nerves. From the polarized-light analysis it is clear that such axon sheaths stand between the thick myelin sheath and the surface membrane of the thin unmyelinated fiber types in respect of lipid-protein ultrastructure. Thus far the surface membrane of "naked" fibers, such as C fibers, has resisted analysis by polarized light.

THE SURFACE MEMBRANE

The classical membrane theory assumes that the polarization potential of the resting nerve fiber and its alterations during activity result from preferential permeability to diffusible ions of a thin metastable surface film or membrane. Certain of the electrical properties of the film, such as the *resistance*, capacity, potential difference, and current flow, have been measured in the resting and active fiber, these measurements have been greatly facilitated by the use of squid giant fibers, in which it is possible to insert one electrode directly into the axoplasm (14, 15). As a result of these measurements and of other evidence (16–18), it has become clear that the classical membrane theory, which assumes that the membrane is depolarized during activity owing to loss of normal selective permeability, must

thickness of 2 μ there would be over a hundred of the repeating periods if continuously packed in the sheath. However, considerations of the water content of the myelin suggest that the sheath *may* contain slabs of such organized myelin separated by aqueous channels.

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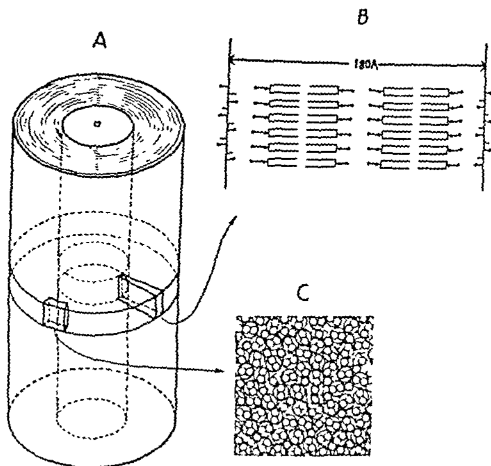


Fig. 1.—Diagrammatic representation of the myelin sheath structure as deduced by polarized light and x-ray *diffraction* studies. *A* shows the lamellar structure of the sheath *B* shows one of the repeating periods in a plane directed radially in the sheath No attempt has been made to show the various types of lipid molecules which form mixed lipid phases The protein bounding the lipid double layers might have been shown as globular particles, since at present there is no evidence concerning the nature of this protein. *C* shows a plane through a lipid layer, i.e., tangentially in the sheath. Small circles indicate individual hydrocarbon chains; large circles indicate the domains of individual lipid molecules

100 A), it should be resolvable with the electron microscope. But by what criteria may any particular interfacial membrane be identified as that which determines the electrical properties?

Unless structural alterations can be demonstrated to result from [illegible] or from activity, it would seem that the ap- [illegible] ly prove most fruitful. [illegible] per have already been [illegible] t lacking in the axoplasm but is concentrated in the "sheath" of the squid giant fiber (21) is an important link in the chain of evidence adduced by Nachmansohn (22) in support of his acetylcholine theory. In the argument the sheath is identified with, or is considered to contain, the metastable "irritable" membrane. In fact, the sheath referred to consists of that portion of the fiber which remains after mechanical extrusion of axoplasm. It includes the "Schwann" cells, the lipid-containing metatropic sheath, connective tissue, and other material adherent to the fiber as usually prepared. Although the chemical estimation of the esterase is doubtless reasonably accurate, the experiment does not closely localize the enzyme. Using histochemical methods, Koelle and Friedenwald (23) and Hard and Peterson (24) find that the reaction for cholinesterase is positive in the Schwann nuclei and the nuclei of glial and connective tissue but negative in the nerve fiber itself.

It has also been claimed that adenosine triphosphatase is located in the "sheath" of the squid giant fiber (25); but this localization is probably even less definitive than that of cholinesterase.

Although histochemical reactions are notoriously capricious and difficult of interpretation even at the level of light-microscope resolution, it would seem to be desirable to attempt to apply the technic to thin sections as viewed in the electron microscope. Investigations of this sort are being undertaken in our laboratory. The choice of substances to be localized and of the type of nerve fiber will be important here. Tests for cholinesterase and adenosine triphosphatase can, of course, be applied. Whether it will be possible to devise a test to demonstrate the sodium complex assumed by Hodgkin and Katz or the quaternary ammonium compound stressed by Lorente de Nó remains to be seen.

THE AXON

The literature on axon structure heavily emphasizes the fibrous structures—the neurofibrils—which can be demonstrated in all nerve fibers after appropriate histological preparation. Depending upon the method of preparation, the fibrils may be relatively coarse, or they

be abandoned. According to the "sodium hypothesis" of Hodgkin and Katz (19), the permeability for sodium becomes much greater than that for potassium or chloride ions during activity. Sodium is assumed to combine with an organic (lipid-soluble) carrier and is free to move as sodium ions only when the membrane is depolarized during activity. Some such hypothesis was required by the fact that the action potential of the squid fiber may greatly exceed the resting potential (16, 17) and by the observations on the effect of alteration of the sodium content of the ionic environment on the resting and action potentials. Hodgkin and Katz consider it improbable that processes concerned with the action potential are oxidative in nature, although oxidative metabolism may be essential for the proper functioning of the sodium-transporting mechanism. They further speculate that a specific enzyme-like process may be involved.

Lorente de Nó (20) found that certain quaternary ammonium ions may substitute for sodium. He further extracted a base, presumably a quaternary ammonium compound, from ox brain which has similar action. He suggested that these quaternary ions are synthesized by metabolic reactions in which sodium takes part. Polarization of the membrane involves chemical reactions which cause a change of trivalent to tetravalent nitrogen in the quaternary ammonium bases.

Investigations and hypotheses of this sort are concerned primarily with the electrical effectors of nerve phenomena—the ions (diffusible or bound). They do not reveal direct information about the chemical composition or microstructure of the membrane itself. Nor do the electrical experiments greatly aid in the precise localization of the critical paucimolecular film. For example, injury of "the inner surface of the cell membrane" of the squid giant fiber by the tip of the microelectrode inserted into the axoplasm causes conduction to cease (14, 16). But is the surface referred to at the boundary between the axoplasm and the "Schwann" cells, between these cells and the lipid-containing metatropic sheath, or peripheral thereto?

What is the prospect that morphological studies may shed light on the location, chemical composition, and microstructure of the critical membrane?

Subject to the indeterminacies introduced by fixation and other alterations caused by the procedure, thin-sectioning may be expected, after further technical improvement, to make possible the direct visualization with the electron microscope of the various interfaces of the nerve fiber. If the critical membrane has a thickness similar to that presumed to be characteristic of the plasma membrane (order of

repeated stimulation. Tobias and Solomon (1950) observed a reversible increase in opacity, light scattering, and shrinkage of the axon locally at the anode during polarization, with opposite effects occurring at the cathode. They suggest that the axon colloid is agglomerated at the anode owing to dehydration, while at the cathode solation occurs. The colloidal mechanism of these effects and their relation to processes of impulse propagation remain to be determined.

The application of the electron microscope to the study of axon ultra-structure is in its earliest infancy. Richards, Steinbach, and Anderson (42) observed rather poorly resolved, kinked, or contorted fibrils in unfixed axoplasm of squid giant fibers. De Robertis and Schmitt (43) described a type of dense-edged fibril in electron micrographs of various kinds of nerves after fragmentation with the blendor or by sonic vibration. Though localization to a particular region of the fiber is difficult in such preparations, it was believed that the fibrils, tentatively termed "neurotubules," were axonic constituents. However, subsequent experiments by the author (44) produced [illegible] [illegible] revealed a type of filament quite distinct from that described as "[illegible] tions of sever[illegible] (45). Dense-[illegible] [illegible], comparable to "neurotubules," were only rarely observed, and then only in the connective-tissue sheath. It may be presumed that the dense edges may have been due to the manner of preparation.

Considerable interest attaches to the axon filaments. In formalin- or osmic acid-fixed fibers these filaments are about 100–200 A in width and present a somewhat contorted, nodose appearance. The discontinuities, frequently having a beadlike appearance, [illegible] fairly [illegible] in the [illegible] odicity [illegible]. The filaments have a roughly similar appearance in all types of nerves thus far examined, vertebrate and invertebrate, and presumably form the basis of the neurofibrils of classical histology. They should probably be included in the list, at present very small, of entities which may be considered as least common factors of nerve structure.

The filaments were observed in fixed nerves, and the question as to the role of the fixative must be examined. Do the filaments preexist in fresh axoplasm, or are they produced by a linear aggregation

may be so thin as to be near the resolving power of the microscope. The physiological role of neurofibrils has been the subject of much speculation since the time of their discovery. They have been considered to be the substratum over which the impulse is conducted, the substratum over which trophic influences pass peripherally from the cell body, and also the lattice which provides mechanical support for the fiber. However, not only is there practically nothing known about the chemical composition of the fibrils, but their very existence in the fresh, unfixed axon is still debatable. The axon in most intact, fresh fibers is optically empty, even in polarized light and in the dark-field microscope. Typical neurofibrils have been demonstrated in unfixed nerve cells in tissue culture (26, 27), and they may make their appearance after peripheral nerve fibers are allowed to stand for some time in artificial media. Ion imbalances may also cause neurofibrils to appear fairly promptly (28). However, in reviewing the literature up to 1929, Peterfi (29) came to the conclusion that the fresh axon is essentially a rodlet sol, which may readily be converted into a fibrous system by the action of reagents. The particles of this rodlet sol were considered to be involved in the propagation of the impulse, although no physicochemical mechanism was proposed for the process. Subsequently, suggestions involving dipole and other types of resonance phenomena along continuous polypeptide chains have been made (30-32).

Polarization optical investigations have confirmed the view that the fresh axon contains asymmetric submicroscopic particles oriented parallel with the fiber axis (33). It was deduced that the partial volume of the fresh axon occupied by these oriented particles is very small (less than 1 per cent). No change in the orientation of the particles, as manifested by a change in birefringence, during impulse propagation was detected (34). Changes in the state of axoplasm with activity have long been sought, but only a few positive results have been recorded. Changes in fiber contour have been claimed following passage of a polarizing current; the region at the anode flattens, while that at the cathode swells (35, 36). More recently, Flaig (37) found that the axoplasm of the squid giant fiber becomes more turgid, failing to flow out from a cut end, when the nerve is stimulated. He believed that excitation shifts the equilibrium from sol to gel. Changes in the absorption of light at specific wave lengths have been observed in stimulated nerves by Lüthy (38) and by Arvanitaki (39). Hill and Keynes (40) have recently detected changes in opacity of the nerves of the walking legs of the shore crab, *Carcinus maenas*, as a result of

mine the relation of the isolated fractions to the resolvable structures of axoplasm.

Only by such a multilateral chemical, physical, and morphological approach may we hope eventually to understand the organization of the nerve fiber and its role in the physiological processes of nerve. The neurophysiologist is likely to regard the analysis of the structure and chemical composition of neuron components as desirable but unlikely to lead very dir[illegible]
is less interested in "[illegible]
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arbitrary. When an enzyme is definitively localized at a particular region, the question of what happens there is at least partially answered. All methods of approach are necessary and provide essential links in the chain of knowledge. Electrophysiologists have provided a few facts and a number of intelligent guesses about the nature of the conducting substratum. As will have become evident from the foregoing, much needs to be done on the chemical and structural side before we shall be in a position to state what is "really there" rather than what "might be there." The necessary technics are fairly well developed, and it remains chiefly to set about getting the work done.

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of particles under the influence of the fixative? Experiments on unfixed axoplasm of *Loligo* and *Myxicola* fibers dispersed in water provide some information on this point. In such preparations there are observed filaments about 100–150 A in width and having smooth edges. These may be assumed to correspond to the contorted and nodose filaments seen in fixed axons. When fresh axoplasm dispersed in water is treated with formalin, nodose filaments similar to those found in formalinized axons are produced.

However, the ionic environment of normal axoplasm, particularly in marine nerves, is very different from that used in isolating the filaments, and this may be expected to influence the appearance and properties of the filaments. When squid axoplasm is extruded into distilled water, the gelled cylinder retains its consistency for 10–30 minutes, whereas, when extruded into sea water or solutions of relatively high ionic strength, the axoplasmic cylinder disintegrates fairly rapidly. It is therefore necessary to investigate the effect of pH, ionic strength, and specific ions upon the constituents of axoplasm, in order to determine whether the filaments are reactive to their environment and whether they pre-exist in normal axoplasm. Experiments along these lines are planned for the near future.

The chemical composition of the axon filaments is unknown. Since the filaments have similar appearance in all the nerve types thus far examined, it is possible that a common protein or protein complex characterizes the fibrous components of nerve axons generally. Although no detailed chemical characterization of axon proteins has yet been made, there is some evidence that a common axon protein or protein complex exists. Bear, Schmitt, and Young (46) described a protein complex obtained from the axoplasm of squid and lobster nerves. Since this material has properties similar in some respects to those of proteins isolated from mammalian central nervous system (47), the possibility was suggested that it may be characteristic of nerve axons generally and was therefore called "neuronin." However, the relation between neuronin and the axon filaments observed in the electron microscope is unknown.

To gain further information about axon proteins, efforts are being made in our laboratory to isolate and purify individual proteins from lobster nerves by modern methods of fractionation. Dr. M. Maxfield has already isolated several fractions. Electrophoresis and ultra-centrifuge data indicate that one fraction is monodisperse and represents a single protein component. Electron-microscope investigations of these fractions are being made by Dr. M. Jakus, in order to deter-

MOTION PICTURE OF NEURONS AND NEUROGLIA IN TISSUE CULTURE

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[Copies of the film are expected to be ready in the near future for rental (in the United States only) and for sale (generally) by the Wistar Institute of Anatomy and Biology, Philadelphia 4, Pennsylvania.]

INTRODUCTION

THE motion picture of neurons and neuroglia was made by Dr. Margaret Reed Lewis and myself at the Department of Embryology, Carnegie Institution of Washington It is the beginning of what we hope some day to expand into a more comprehensive film of living neurons and neuroglia in tissue cultures.

The movements which neurons and neuroglia undergo in tissue cultures and which are exaggerated in the motion picture indicate that neurons and neuroglia, like other types of cells, have a superficial gel layer and less viscous endoplasm and that the gel layer is continuous over cell body, axon or cell process, pseudopod, terminal filaments and [illegible] they would not be noticeable without speeding up, are due to local variations of the contractile tension of the superficial gel layers of the cells.

GEL LAYERS; GENERAL CONSIDERATIONS

The gel layer cannot usually be distinguished optically from the underlying endoplasm in the somatic cells of multicellular organisms. It can, however, be easily seen in many undivided eggs and large blastomeres and in such organisms as amoebae and slime molds because their gel layers are thicker and more viscous than those of small somatic cells. It is from observations and experiments on the gel layers of eggs and amoeboid organisms and the comparison of their behavior with the behavior of somatic cells that one arrives at the conclusion that the latter must also have gel layers.

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by the endoplasm. There is no evidence that migrating cells expand and contract over and over again as they move about.

Contractility, the most important property of gel layers, presumably varies in strength with the viscosity and thickness of the gel layer and the chemical constitution of the cytoplasm. The velocity of contraction, which plays an important role in the rapidity of pseudopod protrusion and retraction, of cell locomotion, and of many early developmental processes, is dependent to a great extent on the chemical constitution of the cytoplasm, on local differences of the strength of the gel layer, and on the viscosity of the endoplasm.

VISCOSITY OF ENDOPLASM

The viscosity of the endoplasm also plays an important role in the rate of movements produced by contraction of the gel layer. As a general rule, cells with fluid endoplasm change form and move more rapidly than those with more viscous or semigelated endoplasm. When endoplasm is in the gel or semigel state, it also exerts contractile tension. When in the gel state, there are indications that it is more viscous peripherally than centrally. The variations of contractile tension as a result of this viscosity gradient are probably responsible for forcing inclusion bodies toward the centrosome, which tends to occupy the center of the cell.

LOCAL INCREASES AND DECREASES OF VISCOSITY

Local increases and decreases of the viscosity and/or thickness of small areas of the gel layer result in local increases and decreases of the contractile tension of the area. Without such local changes, there would be no changes of *form or cell* movements or flow of endoplasm.

THE MOTION PICTURE

The motion picture was made on 35-mm. negative film and the 16-mm. print from it The magnification given in the Table is the magnification in diameters on the 35-mm. negative. When the 16-mm. print is projected, [illegible] screen times the m[illegible] ters of the image [illegible] screen. Thus, if the projected frame is 60 inches wide, the [illegible] diameters, for the scenes [illegible] The speed given equals [illegible] when the film is projected at 960 frames per minute, the normal rate for silent films.

Visible movements of axons and their branches may be roughly classified as follows: axon elongation; waving of terminal membra-

Gel layer and endoplasm are different physical states of the same cytoplasm which more or less readily change from one to the other in the regions where they grade into each other. The superficial aspect of the gel layer is more viscous than its deep aspect, which grades more or less abruptly into the less viscous endoplasm. Cells probably always have a gel layer, even when they are adherent and flattened against one another or against a substratum. Gel layers are a living part of the cytoplasm. They are not static structures; they are always active.

The thickness and viscosity of gel layers and the viscosity of the endoplasm presumably differ, depending on the chemical constitution of the cytoplasm and on external and internal environmental factors. There are many degrees of viscosity of the cytoplasm (gel layer and endoplasm).

The factors responsible for gel-layer and endoplasmic viscosities are obscure. There are indications that gel-layer and endoplasmic viscosity may be the resultant of two factors, an external environmental one which produces a gelating, and a central (centrosomal?) one which exerts a solating, effect. The viscosity at any particular moment at any particular place will depend on a temporary balance between these two antagonistic factors. There are probably other factors involved that have to do with local metabolic processes of the cytoplasm which affect viscosity.

PROPERTIES OF GEL LAYERS

Gel layers are always exerting contractile tension, a fundamental property of protoplasm when in the gel state. In addition to contractility, gel layers are flexible, stretchable, and often adhesive. Contractility is the motive force responsible for changes of cell form, protrusion and retraction of pseudopodia, elongation of axons, cell locomotion, and the streaming flow of endoplasm. It is obvious that flexibility and stretchability are essential for the above changes and that adhesion to a substratum is essential for locomotion. Cells are so small and light that friction with a substratum does not suffice for locomotion—hence their adhesiveness.

Elasticity has frequently been considered the motive force and principal property of gel layers. Elasticity has been much confused with contractility. Elasticity and contractility are entirely different properties. Gel layers are contractile and probably not elastic or only slightly so. The movements which cells exhibit are not due to elastic recoil after previous stretching of the gel layer by inhibition of fluid

AXON PLEXUSES

Most axon outgrowths contain many fibers which cross and adhere to one another by their main stems and by numerous branches, to form irregular plexuses of various densities. As axons elongate, they frequently cross one another at various angles or adhere and run closely parallel for varying distances before separating. Sometimes they fail to separate and form compound axons. As axons elongate, they usually send out new lateral branches, which join other axons or their lateral branches. Part of the terminal pseudopod may also become lateral and have one or several branches. Older lateral branches frequently retract and become disconnected from neighboring ones. The plexus pattern also continually changes by movements of various sorts, such as changing curves of axons and of pulls which a contracting or relaxing filament may have on another one to which it is attached. The frequent production of sharp curves in a filament to the apex of which another filament is attached suggests that such a changing curve is produced by contraction (longitudinal shortening) of the branch or attached filament. There are several examples in scene 1.

If one assumes that an axon and its branches have an outer gel layer and an inner, less viscous core, that the gel layer is always exerting contractile tension in various directions, and that the contractile tension is subject to many local variations, then one would expect continual changes in any plexus formed by them.

It seems ...

... giant cells. Fibroblasts may have long branching processes of various sorts which come into contact with neighboring fibroblasts and their processes and adhere without fusion. The withdrawal of axon branches from contacts with neighboring axons and the usual separation of two axons which were in close contact for some distance and the elongation of each in a different direction demonstrate adhesion, not fusion.

There are indications that branches of the same axon may actually fuse. Scene 17 shows the fusion of part of a ...

... with varying degrees of adhesiveness. The fact that they frequently bend and sway indicates that adhesion is not excessive.

nous pseudopodia; increases and decreases of axon diameters; bendings of axons and branches; protrusion, elongation, and retraction of branches; local bulges, which look like granules, and their movements back and forth; and the scintillation effect.

TABLE OF SCENES IN MOTION PICTURE*

Scene	Mag.	Spd.	ET	PT	Comments
	Intestine, 7-Day Chick Embryo, 48-Hour Culture, Fluid Medium, LBD				
1-41	85	204	568	165	Sympathetic nerve plexus
2-671*a*	200	24	15	40	2 nerve cells, 1 microglia
3-671*b*	200	24	15	40	4 axon ends
4-673*a*	200	24	22	58	Mass of adherent axons, dense plexus
5-673*b*	200	24	13	33	Axons, fibroblast with dark, wavy edge
6-680	200	40	20	32	*Single axon, terminal cone, and pseudopod*
	Brain, 7-Day Chick Embryo, 2½-3½-Hour Culture, Fluid Medium, LB				
7-233	44	85	242	169	Oligodendroglia? Threadlike
8-234*a*	200	85	30	20	Field shifts
9-234*b*	200	85	27	19	Field shifts
10-234*c*	200	85	21	15	Field shifts
11-234*d*	200	85	26	17	Field shifts
	Spinal Cord and Medulla, 11-Day Chick Embryo, 24-Hour Culture, 1 LBD—1 Cp				
12-672*a*	200	24	15	37	Axons, terminal cone, and pseudopod
13-672*c*	200	24	12	30	Axons, local bulges move back and forth
	Spinal Cord, 7-Day Chick Embryo, 48-Hour Culture, LBD				
14-677	200	24	13	33	Mass of adherent axons, dense plexus
	Spinal Ganglion, 8-Day Chick Embryo, 48-Hour Culture, 1 Cp—1 Ce				
15-715*a*	200	24	19	49	Axon, terminal cone, and pseudopod
16-715*b*	200	8	5	47	Pseudopod membrane, waves
17-715*c*	200	24	16	42	Pseudopod membrane, waves
	Brain, 17-Day Mouse Embryo, 24-Hour Culture, L—Cp—Ce—FS				
18-681*a*	200	40	24	37	Astrocytes? Peculiar lobulations
19-681*b*	200	40	25	36	1 astrocyte, 2 microglia
20-681*c*	200	40	21	33	Mitosis
21-681*d*	200	40	29	43	Astrocytes, wavy pseudopod

* *Abbreviations:* ET = actual elapsed time in minutes from the beginning to end of scene, PT = the projection time in seconds at normal projection rate, L = Locke solution, B = chicken bouillon, D = dextrose, Cp = chicken plasma, Ce = chick embryo extract; FS = fetal blood serum.

AXON PLEXUSES

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tensior [illegible]

[illegible]

[illegible] As a general rule, other types of cells seen in cultures retain th[illegible]

ditions to form sy[illegible]

may have long bra[illegible] processes of various sorts which come into contact with neighboring fibroblasts and their processes and adhere without fusion. The withdrawal of axon branches from contacts with neighboring axons and the usual separation of two axons which were in close contact for some distance and the elongation of each in a different direction demonstrate adhesion, not fusion.

There are indications that branches of the same axon may actually fuse. Scene 17 shows the fusion of part of a t[illegible]

nei[illegible]

[illegible]

pi[illegible]

[illegible] The [illegible]

adhesion is r[illegible]

AXON ELONGATION

The motion picture shows numerous examples of axon elongation but no clear demonstration of its mechanics. Two theories have been suggested: (1) that the axon is pulled and stretched by the advance of the amoeboid tip and (2) that the axon is elongated by material which is squeezed from the cell body along the axon to its tip, where it advances the terminal pseudopod and gels at its sides to elongate the axon gel tube.

Harrison (1910) states that "the free end of each fiber is enlarged and provided with fine processes or pseudopodia. This part continues its progression and the fiber is gradually drawn out." The "ductility of the ectoplasm is such, that the movement results in the formation of long fibers, the primitive axones." The idea that the amoeboid end moves along under its own steam or is pulled along by surface-tension forces and that this stretches the axon is in agreement with the idea which some authors have regarding the locomotion of free cells, but it is not in agreement with what happens during the elongation of a free pseudopod of an amoeba.

In 1945 I suggested that the gel layer of the axon is lengthened (elongated) by the gelation at its distal end of endoplasm, which is *forced from the cell body along the axon by contraction of the gel* layer of the cell body, and that the mechanism is similar to pseudopod elongation of the amoeba. This idea seemed to be supported by the observation that elongation occurs on the distal side of the cross-over point of two axons. Such axons adhere at the cross-over point; yet they are not bent distally, as one would expect if axons are lengthened by pulls at the distal end. It was also found that elongation always occurred on the distal side of the lateral branches and that the latter remained fixed in position with reference to an outside point. Lateral branches were not pulled along. I also suggested that this theory offers an explanation for the damming effects obtained by Weiss (1944) when nerves are constricted (cf. Weiss and Hiscoe, 1948). The continued or perpetual forcing of endoplasm distally along the axon gel-layer tube by the contraction of the gel layer of the cell body would result in the accumulation of endoplasm proximal to the "bottle neck" and in distortion of the axon.

THEORY OF AXON ELONGATION

Axon elongation occurs at the tip by the addition of cytoplasm. Just how this is accomplished has not been observed because the tip is too minute and the process too slow for the eye to unravel. My

theory of axon elongation is based on observable events that occur during the elongation of pseudopodia of *Amoeba proteus* and *Chaos chaos*.

The gel layer of the neuron extends over cell body, axon, and pseudopodal tip. It is always exerting contractile tension. The gel layer of the pseudopod is weaker than that over the rest of the cell and axon. Continued contraction of the gel layer of the cell body exerts pressure on the less viscous endoplasm, which is pressed against the entire cell wall. The weakest part—the weak gel layer of the terminal pseudopod—is expanded and advanced. The contraction of the gel layer over the body of the cell forces endoplasm out of the body and along the axon into the pseudopod and expands it. Endoplasm is deflected to the sides at the base of the thin-walled pseudopod and gels. This progressively elongates the gel wall of the axon. The thin-walled pseudopod is wider than the axon. As endoplasm gels at the base of the pseudopod, it thickens the gel layer, and the latter begins to exert increased contractile tension and constricts the wall to axon size. Contractile tension of the gel wall of the axon also plays a role in forcing or squeezing endoplasm against the weak gel layer of the terminal pseudopod.

THE FLOW OF ENDOPLASM IN AXONS

The d[illegible]
very sl[illegible]
upon th[illegible] of granules and mitochondria, as the endoplasm itself is almost invisible. Matsumoto observed back-and-forth movements of small, neutral, red-stained granules and mitochondria, but no constant distal movement. At that time no one had any idea of a distal flow, and hence he did not look for it.

The mechanics of endoplasmic flow in such minute tubes would be something of a problem if the tubes were rigid, as the pressure necessary would presumably far exceed the contractile tension of the gel layer of the cell body The axon wall or gel layer, however, is always in a state of contractile tension. It exerts pressure on the endoplasm along its entire length and that of its branches and forces endoplasm against a weak area. This, combined with the contractile tension exerted by the gel layer of the cell body, gives a mechanical setup which meets the difficulties of a rigid tubular system.

COMPENSATORY GELATION OF PSEUDOPOD GEL LAYER

It is obvious that the weak, thin [illegible]
[illegible]

tory increase in its thickness. I suspect that endoplasm gels on the inner aspect of its gel layer as rapidly as the latter is thinned by stretching. Some gelating factor in the external environment may be responsible.

Perplexing problems arise the moment one begins to consider what factors may be responsible for the continued weakness of the gel layer at the tip, as contrasted with the progressive gelation of the endoplasm at the adjacent base of the weak area.

THE TERMINAL CONE

The presence of a terminal cone indicates a progressive increase of contractile tension of its gel layer. The gel layer of the cone is presumably thicker where it merges into axon than where it merges into pseudopod, with corresponding differences of strength of its contractile tension. It takes time for the newly formed gel to develop contractile tension of axon strength: the longer the cone, the longer the time. Cones are sometimes very short or absent, and sometimes they persist for a long time as local spindle-like enlargements of an axon some distance from the tip.

TERMINAL PSEUDOPODIA

Terminal pseudopodia have usually been pictured as consisting of several terminal filaments which extend directly from a terminal cone or a thin terminal expansion or an unexpanded end (Harrison, 1910; Lewis and Lewis, 1912).

The motion picture shows only a few axon ends. Most of them seem to consist of several dark terminal filaments which exhibit peculiar wavy movements. I suspect that they are edgewise views of folds of a terminal undulating membrane because they behave like similar ones in a terminal membranous pseudopod of the axon shown in scenes 16 and 17. Similar dark, waving edges are frequently seen on other types of cells, in which a wavy, membranous pseudopod curls and folds so as to present edgewise views. Edgewise folds cut out part of the light and appear dark. Sometimes the dark edgewise view of a fold appears to be discontinuous. They move more rapidly and differ enough in appearance to indicate that they are different from lateral filaments. The terminal membranous pseudopod is difficult to see and to photograph.

One can, however, find numerous examples on fixed and stained cultures. The membrane is thin and pale and easily overlooked if not stained deeply enough. The stained cultures of sympathetic plexuses

show more and longer lateral filaments than are detectable in the film. They also show some exceedingly long, terminal, single filaments which are just on the limits of visibility with a 2-mm. lens and also some ends which seem to have several terminal filaments.

The general principle involved in elongation is the same for axons ending in one or several terminal filaments as for ones ending in a thin, wavy membrane. Even the smallest filament and its branches have a gel layer and a less vicous core. Those that seem to end abruptly are more like those of the amoeba than are those ending in a wavy membrane.

Since all the axons examined were more or less adherent to the cover glass, one wonders whether surface-tension forces may be concerned with the spreading of the tips into a thin membrane. Such spreading is not essential for axon elongation and could not by itself elongate the axon.

A membranous pseudopod probably has a gel layer on each side, which is continuous over its edges, and a central, less viscous core. The latter is probably gelated enough to bind the outer gel layers to it and thus to prevent them from bulging out into a globule as fluid endoplasm is squeezed into the meshes of the core.

WAVING OF TERMINAL MEMBRANOUS PSEUDOPODIA

The wavy motions of terminal pseudopodia are similar to those which occur on other types of cells, except that the foldings tend to radiate from the end of the axon. All the movements are probably produced by local changes in the viscosity of the gel layer. Little increases and decreases cause the membrane to bend or curl in various ways.

The ruffle-like pseudopodia of macrophages, fibroblasts, and sarcoma cells [illegible] of the surrounding [illegible] This same drinking process can be seen in scenes 16 and 17. This also indicates that membranous axon pseudopodia are similar to those of other somatic cells.

The factors responsible for the minute local changes of contractile tension of the gel layer which result in the waving motion are obscure. They can scarcely be attributed to changes going on in the cell body, since it may be far removed from the axon tip, or to local differences in the external environment. Perhaps metabolic changes in the gel layer and endoplasm which are probably nonhomogeneous may be responsible for the minute local changes in thickness and/or viscosity and hence the minute local variations of contractile tension.

PROTRUSION, ELONGATION, AND RETRACTION OF AXON BRANCHES

Lateral filaments are frequently protruded from the main axon stem, especially near the distal end. Such filaments may extend for long distances. They, in turn, may send off branches. Lateral filaments may also retract. It has already been suggested that all filaments, no matter how minute, have an outer gel layer and a less viscous endoplasmic core.

They presumably arise at a minute local weak area, which protrudes as the contraction of the gel layer over the rest of the cell forces endoplasm against the weak area of the gel layer. They will continue to elongate as long as the tip remains weak, in the same manner as an axon or an amoeba pseudopod elongates, namely, by gelation of the endoplasm at the base of the tip to prolong the gel-layer tube. The thin, weak tip is presumably kept from becoming too thin by a compensatory gelation of endoplasm on its inner aspect.

Retraction will ensue if the gel layer of the tip increases sufficiently in thickness and/or viscosity to exert enough contractile tension to force the endoplasm backward. As the gel layer of the tip and lateral wall exerts increased contraction, it shortens, and its inner aspect solates and joins the endoplasm, just as with a retracting pseudopod of *Amoeba proteus* or *Chaos chaos*. The same principle is presumably involved in each case.

BENDINGS OF AXONS AND THEIR BRANCHES

Some axons elongate in nearly straight lines; many elongate in curves, sometimes in arcs of 90°–180°. The fact that they often elongate in curves would seem to eliminate the idea that they are pulled out by an amoeboid tip. Most axons and their branches show local bendings of various dimensions that slowly change. These changes are more evident when motion is speeded up in the film. It seems probable that these changing curves and bends are due to local increases and decreases in the longitudinal contraction of the gel layer. It seems rather far-fetched to assume that the longitudinal contraction of the gel on one side of an axon or on one side of one of its small branches could be greater than that on the other side. We are dealing with minute things so far as the eye is concerned, but with enormous giants from a molecular standpoint.

Some of the bendings seen in the film seem to be due to shortening or elongation of an attached branch. The apparent pull exerted by a contracting branch or attached filament may produce quite a sharp curve in the filament.

INCREASES AND DECREASES OF AXON DIAMETERS

Axons differ in diameter from about ½ to 1½ μ. Their branches are much smaller, some at about the limits of visibility with a 2-mm. objective. Single axons also show considerable variation in different regions and slow regional increases and decreases. They frequently have local enlargements where lateral branches arise. The enlarged terminal cones have already been considered.

The special factor responsible for the small and fairly uniform diameter of an axon is the fairly uniform circular contractile tension *of its gel layer*. The orientation of the contractile tension of the gel layer is the peculiar feature. The gel layer exerts contractile tension in other directions, and there are indications of longitudinal tension also. *The* circular orientation predominates. This is probably determined by the peculiar chemical constitution of *neuron* cytoplasm, which somehow causes a special orientation of its molecules as it gels.

LOCAL BULGES OF AXONS AND BRANCHES AND THEIR MOVEMENTS

As axons degenerate, they usually develop local bulges and become beaded, presumably because of local decreases in gel-layer thickness and/or viscosity. These small, local bulges appear darker than the rest of the axon, presumably because of refraction and diminution of light.

Somewhat similar bulges occur on axons that are still in fairly good condition. They were at first mistaken for large, dark granules which were moved back and forth along a fiber, causing it to bulge. There is one place in scene 13 where the pseudo-granules appear to be moving in opposite directions in the same axon. Further analysis revealed that there were two axons close together, each one with its own bulges. Each bulge retains its own identity. The rate of movement is much exaggerated in the film.

Bulges occur at weak areas of the gel layer because the somewhat greater contraction over the rest of the axon forces endoplasm against the weak areas and expands them.

How do the bulges move back and forth along the fibers? Several explanations have been considered: (1) that local weakened areas of the gel layer develop in a wavelike manner along the axon; (2) that the endoplasmic contents of the bulge are somewhat gelated and that local increases and decreases of the contractile tension force the lump along the axon; (3) that longitudinal increases and decreases of the contractile tension of the gel layer pull the bulges.

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Retraction will ensue if the gel layer of the tip increases sufficiently in thickness and/or viscosity to exert enough contractile tension to force the endoplasm backward. As the gel layer of the tip and lateral wall exerts increased contraction, it shortens, and its inner aspect solates and joins the endoplasm, just as with a retracting pseudopod of *Amoeba proteus* or *Chaos chaos*. The same principle is presumably involved in each case.

BENDINGS OF AXONS AND THEIR BRANCHES

Some axons elongate in nearly straight lines; many elongate in curves, *sometimes in arcs of 90°–180°. The fact that they often* elongate in curves would seem to eliminate the idea that they are pulled out by an amoeboid tip. Most axons and their branches show local bendings of various dimensions that slowly change. These changes are more evident when motion is speeded up in *the film*. It seems probable that these changing curves and bends are due to local increases and decreases in the longitudinal contraction of the gel layer. It seems rather far-fetched to assume that the longitudinal contraction of the gel on one side of an axon or on one side of one of *its small branches could be greater than that on the other side*. We are dealing with minute things so far as the eye is concerned, but with enormous giants from a molecular standpoint.

Some of the bendings seen in the film seem to be due to shortening or elongation of an attached branch. The apparent pull exerted by a contracting branch or attached filament may produce quite a sharp curve in the filament.

INCREASES AND DECREASES OF AXON DIAMETERS

Axons differ in diameter from about $\frac{1}{3}$ to $1\frac{1}{2}$ μ. Their branches are much smaller, some at about the limits of visibility with a 2-mm. objective. Single axons also show considerable variation in different regions and slow regional increases and decreases. They frequently have local enlargements where lateral branches arise. The enlarged terminal cones have already been considered.

The special factor responsible for the small and fairly uniform diameter of an axon is the fairly uniform circular contractile tension of its gel layer. The orientation of the contractile tension of the gel layer is the peculiar feature. The gel layer exerts contractile tension in other directions, and there are indications of longitudinal tension also. The circular orientation predominates. This is probably determined by the peculiar chemical constitution of neuron cytoplasm, which somehow causes a special orientation of its molecules as it gels.

LOCAL BULGES OF AXONS AND BRANCHES AND THEIR MOVEMENTS

As axons degenerate, they usually develop local bulges and become beaded, presumably because of local decreases in gel-layer thickness and/or viscosity. These small, local bulges appear darker than the rest of the axon, presumably because of refraction and diminution of light.

Somewhat similar bulges occur on axons that are still in fairly good condition. They were at first mistaken for large, dark granules which were moved back and forth along a fiber, causing it to bulge. There is one place in scene 13 where the pseudo-granules appear to be moving in opposite directions in the same axon. Further analysis revealed that there were two axons close together, each one with its own bulges. Each bulge retains its own identity. The rate of movement is much exaggerated in the film.

Bulges occur at weak areas of the gel layer because the somewhat greater contraction over the rest of the axon forces endoplasm against the weak areas and expands them.

How do the bulges move back and forth along the fibers? Several explanations have been considered: (1) that local weakened areas of the gel layer develop in a wavelike manner along the axon; (2) that the endoplasmic contents of the bulge are somewhat gelated and that local increases and decreases of the contractile tension force the lump along the axon; (3) that longitudinal increases and decreases of the contractile tension of the gel layer pull the bulges.

SCINTILLATION EFFECT, MINUTE MOVEMENTS

All axons show minute movements, presumably within the gel layer, as though there were minute alternating changes of viscosity everywhere in the gel layer at all times. They produce a scintillation effect, in that they seem to affect the transmission of light through the axon. These minute changes of viscosity may be more fundamental than the larger local changes responsible for bends and elongation. They indicate that the gel layer is in a constant state of activity.

NERVE CELLS

Some of the scenes show a few nerve cells which are entangled in the nerve-fiber plexuses. They are still capable of migrating a little and cause considerable disturbance of the plexuses as they move. Some of them seem to degenerate.

FIBROBLASTS

Most scenes also show relatively large, flat, migrating fibroblasts with wavy, membranous pseudopodia which present curving, dark, edgewise views of this curling membrane. Such edges are darker than the rest of the membrane and support the idea that axons also have terminal membranes which curl in various ways and present edgewise views which look like terminal filaments.

OLIGODENDROGLIA(?)

The motion picture has several scenes showing the migration of *exceedingly long, threadlike cells from the brain of a 7-day chick* embryo cultivated in a fluid medium. The exact identity of the cells is uncertain, but they seem to be like oligodendroglia. They begin to migrate within 2–3 hours after a culture is set up and move in rather *straight radial lines. In the course of 4 hours after the beginning of migration, great numbers are present. The relatively large nucleus* produces a marked bulge. The cytoplasm tapers abruptly at both ends of the nucleus into the long, slender, threadlike processes. Most of the cells are bipolar; an occasional tripolar one can be seen. Vital stains made on other preparations show mitochondria, neutral red granules, and occasional fat globules. The nucleus often undergoes various contortions.

ASTROCYTES(?)

Cultures of the brain of a 17-day mouse embryo show, in addition to many axons, a few large, peculiar, lobulated cells, which we have provisionally designated as "astrocytes." Some of them have a small,

wavy, membranous pseudopod on the slender tip of a long cell process. They resemble axon pseudopodia. Many of the lobulations seem to contain a large, dark granule. They also show movements of granules in the endoplasm.

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Satisfactory direct observations on nerves in the living animal are possible a short time after hatching. At this time the tail fins develop rapidly and soon become thin and transparent enough for good microscopic visibility. Observations *in vivo* may then be carried on up to the time of the onset of metamorphosis.

Nerve-fiber changes may be conveniently considered in this account under the following headings: (1) adjustments of the early nerve sprouts, (2) adjustments of sheath cells, myelin segments, and end-arborizations, and (3) correlated adjustments of nerves and special sense organs.

II. ADJUSTMENTS OF THE EARLY NERVE SPROUTS

A. PIONEER NERVE FIBERS

[illegible] the [illegible] (gr[illegible] cone) of a pioneer nerve fiber advances slowly through the loose mesenchymal tissue by a typical amoeboid motion. Its progress is strikingly portrayed by ciné-photomicrographs of the fast-motion type. Delicate filamentous processes are extended and retracted. The speed of advance is rather variable. A rapidly growing tip may advance at the rate of about 60 [illegible] the tissues and grounc[illegible] A favorable position f[illegible] advance of a growing nerve tip appears to be a zone immediately below the basement membrane of the cutaneous epithelium. The commonest obstacles it encounters are the processes of young connective-tissue cells which may lie across its path. Blocking is followed by a damming-up of neuroplasm and enlargement of the tip. Branching may then take place at or near the tip. Such branching may be either temporary or permanent. Sometimes a certain amount of retraction may precede the branching, or retraction and growth in a new direction without any branching may occur. Rarely a length of the nerve tip after prolonged blocking may be pinched off (nerve autotomy) and suffer degeneration.

Just what factors determine the exact path taken by a pioneer nerve tip in its advance from moment to moment is not entirely clear.

ADJUSTMENTS OF PERIPHERAL NERVE FIBERS

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I. INTRODUCTION

IT IS possible to study individual nerve fibers in living frog tadpoles by direct microscopic observation. Many *in vivo* studies of this kind have been made by the writer during the last twenty years (Speidel, 1932–49).[1] The changes during growth and regeneration, as well as those during various degrees of injury and recovery, have been observed from day to day for long periods of time. A great deal of flexibility of adjustment on the part of the nerve fibers has been noted. In this account I shall call attention to some of the adjustments exhibited by the peripheral nerves of tadpoles under both normal and experimental conditions.

For microscopic examination, an animal is slightly anesthetized with weak chloretone and placed in a special glass observation chamber. The tail tip of the tadpole is oriented and held in a favorable position, and the chamber is then placed on the stage of the microscope. The microscope tube is tilted nearly to a horizontal position. Nerve fibers in the tail fins may be watched under high magnification. The animal can be kept alive for further observations on the same nerve fibers during the following days.

Changes in nerves are best recorded by ciné-photomicrography (Speidel, 1935*a*, *b*, 1946). Motion pictures are taken at either the normal rate or at slow speeds. Pictures of the latter type depict all movements greatly accelerated. They reveal rather vividly the slow movements of nerve fibers and adjacent tissues. Motion pictures constitute excellent permanent records of the various adjustments of nerve fibers during growth and regeneration.

The great majority of nerve fibers in the tail fins are those which convey impulses of general cutaneous sensation. They are the periph-

1. During the period from 1932 to 1949 this work was aided by grants from the following: National Research Council (Committee on Grants-in-Aid), American Medical Association (Committee on Scientific Research), Penrose Fund of the American Philosophical Society, and the American Cancer Society (Committee on Growth).

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II. ADJUSTMENTS OF THE EARLY NERVE SPROUTS

A. *PIONEER NERVE FIBERS*

Shortly after hatching, the first nerve sprouts may be discerned in the tail fins as they grow out toward the skin. The growing tip (growth cone) of a pioneer nerve fiber advances slowly through the loose mesenchymal tissue by a typical amoeboid motion. Its progress is strikingly portrayed by ciné-photomicrographs of the fast-motion type. Delicate filamentous processes are extended and retracted. The speed of advance is rather variable. A rapidly growing tip may advance at the rate of about 60 μ an hour. Its course is influenced by the tissues and ground substance of the terrain in which it is growing. A favorable position for the advance of a growing nerve tip appears to be a zone immediately below the basement membrane of the cutaneous epithelium. The commonest obstacles it encounters are the processes of young connective-tissue cells which may lie across its path. Blocking is followed by a damming-up of neuroplasm and enlargement of the tip. Branching may then take place at or near the tip. Such branching may be either temporary or permanent. Sometimes a certain amount of retraction may precede the branching, or retraction and growth in a new direction without any branching may occur Rarely a length of the nerve tip after prolonged blocking may be pinched off (nerve autotomy) and suffer degeneration.

Just what factors determine the exact path taken by a pioneer nerve tip in its advance from moment to moment is not entirely clear.

Mechanical, electrical, and chemical factors have been proposed by various investigators (cf. Harrison, 1935).

Long ago Harrison (1914) gave a convincing demonstration of the importance of mechanical or structural factors. He showed that a spider-web frame placed in a tissue culture with nerve cells markedly influenced the pattern of outgrowing nerve fibers. The fibers exhibited a *definitely positive stereotropism and grew out along the web filaments.*

More recent experiments by Weiss (1934, 1941) have furnished much additional evidence along this line. He emphasizes that submicroscopic structures, as well as ordinary microscopic ones, are important in the determination of the path of nerve growth. He points out that an oriented ultra-structure of a tissue-culture medium, such as a clot, may be experimentally induced by a variety of means and that nerve fibers growing in it merely follow the paths set up in the ground substance by the oriented micellae. To describe this behavior, he proposes the term "contact guidance." The nerve-fiber growth cone in its progression is in actual contact with the oriented lines of submicroscopic structures and is guided by them. The directive factor is immediate, not remote.

Throughout my observations on growing nerves in the living frog tadpole, I have seen nothing in conflict with these views of Harrison and of Weiss. The configuration of the terrain, both microscopic and submicroscopic, immediately adjacent to a growing nerve-fiber tip certainly must be of prime importance in influencing the path of nerve growth. One point may be mentioned in this connection. Fast-motion ciné-photomicrographs of the mesenchymal regions being traversed by growing nerve fibers in tadpoles show that there are constant movements of both cells and ground substance. Cells are not quiescent. On the contrary, they pulsate, divide occasionally, and shift their positions. As a consequence, the ground substance is subjected to changing stresses and strains. Changes occur, therefore, from time to time in whatever directive lines there may be in the terrain in which nerve fibers are growing.

Evidence for and against the view that an electric current may play a role in influencing the path of nerve growth has been brought forward. Marsh and Beams (1946) have discussed this subject recently. They hold that the direction of nerve growth may be definitely affected by electricity. They present photographic evidence which clearly shows how a direct electric current of suitable density may appreciably affect the direction of growth of fibers in an explant of nerve

cells from the medulla of the chick. Growth of fibers was suppressed at the anode but not at the cathode. At other places the fibers grew outward at first and then bent toward the cathode. There was no indication, however, that the fibers followed the lines of force of the current. Marsh and Beams think that these results cannot be explained on the basis of "contact guidance" by submicroscopic micellae oriented by the current. They believe that their results indicate the possibility that electrical forces have an influence in establishing nerve patterns in the living animal.[2]

It is probable also that general chemical factors influence nerve growth and regeneration; it is uncertain, however, whether special neurotropic substances play a part in attracting nerve sprouts to grow in their direction. In the case of a living frog tadpole, a growing nerve sprout may be transformed readily into a retracting one by chemical treatment. Examples of this have been described for alcohol, metrazol, and chloretone (Speidel, 1936, 1940, 1942b). Furthermore, a nerve ending which has retracted some distance may, on recovery, grow out in a new direction, not following the old pathway. The mechanism of action of the chemical solution is not *entirely clear*. The action may be on the nerve ending directly, or it may be on the nerve cell as a whole. In the latter case, retraction of the ending would be a reflection of the stress
that many types of cell
In any case, whatever the mechanism may be, experiments of this sort show that chemical treatment may effect a change in a nerve pattern. There is nothing specific about such an effect. A similar result may be caused by a treatment with electricity or with heat (Speidel, 1942b). On
retraction, its pat
lines of the immed
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Cajal (1928) and many others have been favorably disposed toward the theory of "neurotropism." According to this theory, nerve sprouts are sensitive to special chemical substances; such substances, produced in the neighborhood of growing nerve tips, attract them.[3]

2 A few experiments by the writer on growth cones in tadpoles placed in an electric field yielded only negative results These were regarded as *inconclusive* (Spe...

3. An interesting comm...
tissues appears to furnis
lymph vessel to give off a
blood cells, and retur
lymph endothelium a

Neurotropic substances are thought in this way to influence the nerve pattern that develops.

Weiss and Taylor (1944) have presented some experimental evidence against this theory. They point out that some supposed neurotropic agents, such as degenerating nerve, do not in their experiments exhibit any particular attraction for regenerating nerve fibers or influence the path taken by growth cones. Weiss thinks that some supposed cases of neurotropism may be better explained on the basis of contact guidance.

My work on tadpoles has not yielded any crucial evidence along this line. Certain observations on the nerves and sense organs of the lateral line suggest strongly that these special sense organs may exert a specific tropic influence on the vagus nerve branches that are destined to innervate them and, further, that they do not exert such an influence on the spinal nerve branches in the vicinity that are destined to innervate the skin. If such a tropic influence is actually at work in this instance, it operates over only a relatively short distance. Further observations and experiments on suspected neurotropic mechanisms are desirable.

B. LATER NERVE FIBERS

The primitive nerve lines which are laid down by the pioneer fibers have a decided directive influence on later sprouts. This influence is exerted in two ways. In the first place, the pioneer fibers offer stereotropic aids to the growing tips of subsequently developing fibers. In the living tadpole the second and third growth cones may often be discerned as they slowly make their way along the pioneer fiber, clinging more or less closely to it as they advance. In the second place, the neuroplasm of the later growth cones appears to have a certain affinity for the neuroplasm of the pioneer fiber. To some degree there is a selective adhesiveness between the two. This results in the laying-down of small nerves, each at first consisting of a few naked fibers. At times an advancing growth cone may diverge from the nerve line it is following and grow across to join a neighboring nerve. In this way a plexus may start.

It is of interest to note that the property of "selective adhesiveness" exhibited by the early nerve fibers is not unique for nerve tissue. A blood vascular sprout exhibits a similar selective adhesiveness for other blood vascular sprouts, a lymph vascular sprout for other lymph vascular sprouts, and a fibroblast process for other fibroblast processes. An affinity of this sort between like tissues or cells makes for orderly development.

III. ADJUSTMENTS OF SHEATH CELLS, MYELIN SEGMENTS, AND END-ARBORIZATIONS

Sheath cells migrate out from the central nervous system. They flatten themselves along the young nerves and move slowly peripheralward. From the time of their first appearance, they exhibit a marked affinity for nerve substance. They multiply by mitosis and become spaced at appropriate intervals. They form the nucleated sheath of Schwann. They are also responsible for the differentiation of a delicate, external, limiting membrane (membrane of Schwann). This is a tubular structure which appears to be syncytial; it continues from one sheath cell to the next and incloses the nuclei and soft protoplasm of the sheath cells as well as the incased nerve tissue.

After a certain degree of maturity has been attained, some nerve fibers become ensheathed with myelin. This sheath is laid down in segments, one segment for each sheath cell. Myelination starts centrally and proceeds peripherally. Myelin is a product of the co-operative activity of both nerve tissue and sheath cell. Neither one alone is able to elaborate it (Speidel, 1932, 1933, 1935*a*, *b*).

Myelination is accompanied by a profound change in the nerve fiber. The fiber swells, changes somewhat in its chemical reactions (such as to stains), and acquires alternating node and internode regions. Physiologically, a myelinated fiber conveys nerve impulses faster than an unmyelinated fiber. The myelin sheath also insulates and protects the inclosed nerve fiber.

Collateral end-arborizations develop at some nodes. These in some cases represent modifications of earlier side branches of fibers before myelination; in other cases they arise after the myelin sheath has been formed. They undergo changes from time to time to keep pace with the growth of the neighboring tissues (Speidel, 1942*a*). Indivi-[illegible] ual endings [illegible] retraction. S[illegible] lost. Aberra[illegible] [illegible]cutaneous nerves, which are at first directed toward the deeper tissues instead [illegible] [illegible] [illegible]ments directed toward the surface, after which the older, deeply located branches degenerate. Endings that fail to reach the skin are ultimately eliminated. In general, the resting terminal branches of an end-arborization exhibit adjustments which are much like those of early growth cones. They may also be

modified in pattern by treatments with alcohol, metrazol, chloretone, insulin, electricity, and heat in much the same manner as are the early, growing nerve tips (Speidel, 1942*b*).

It is not clear what starts myelination of a fiber. Certainly, it is not merely a question of age; the oldest fibers of a nerve are not necessarily the ones that first become ensheathed with myelin. One theory states that the assumption of function by a fiber is responsible for its becoming myelinated. This point of view cannot be rigidly maintained, however, since it can be disproved in some instances by direct observations. In certain regenerating fibers, most clearly those of the vagus nerve which normally innervate special sense organs, it can be seen that myelination of fibers may occur before the organs are innervated, that is, before the establishment of any specific function. It may also occur in the case of aberrant fibers that never make successful end-connections, fibers that are destined later to degenerate (Speidel, 1948). My evidence indicates very strongly, however, that successful end-connections of a fiber are essential for the maintenance of its myelin sheath.

Various adjustments of sheath cells and of myelin segments may occur. During the early stages of nerve growth, before the appearance of myelin, the sheath cells move about freely and multiply. They are active in bringing about alignment of fibers, and they aid in the organization of the young nerves. They are responsive to wounds and also to slight irregularities that may affect the nerves on which they are located. They may migrate along a nerve to a site at which some abnormality exists and aid in bringing about suitable adjustments. Specific cases of this have been described and illustrated (Speidel, 1932, 1933, 1935*a*, *b*). A sheath cell, after settling down as part of a myelin unit, moves about much less freely; it is still responsive, however, to any irregularities that affect its nerve fiber. For example, it will assist in the replacement of any portion of the myelin that may be lost, provided that the nerve substance remains alive.

During the process of myelination, sheath cells display a pronounced differential preference. They move from an ordinary unmyelinated fiber to a fiber which has begun to acquire myelin sheath segments. In other words, the sheath cells are attracted by a "myelin-emergent" fiber.

Nerve-sectioning experiments reveal another differential preference on the part of the sheath cells. As is well known, after a nerve is transected, the sheath cells on the distal stump exhibit definite responses. In addition to the early activity of these cells in furthering

digestion and disposal of the degenerating nerve substance and myelin, they multiply and migrate. Direct observations of the distal stump in a tadpole show migration of sheath cells from the cut nerve end into the wound zone, and movements also along the stump. If the stump is re-innervated, the sheath cells again function in the formation of new myelin sheaths on the regenerating fibers. If the stump is not re-innervated, many of the sheath cells transfer to adjacent living nerve fibers. Thus a sheath cell has a greater affinity for living nerve substance than for a degenerating nerve stump, especially one of long standing. Sheath cells that are stranded permanently on nerve stumps devoid of any living fibers display marked regressive changes. In my opinion, there is a reciprocal nutritive relationship between sheath cell and nerve fiber. Each one exerts a marked trophic influence on the other.

IV. CORRELATED ADJUSTMENTS OF NERVES AND SPECIAL SENSE ORGANS

Recent experimental studies by the writer on the regeneration of the nerves and special sense organs of the lateral line have yielded some significant results. These are concerned chiefly with the reciprocal influence of one kind of tissue *on another*. A special sense organ and its specific nerve are organized into a closely knit functional unit. This unit may be readily interfered with experimentally and the resulting structural changes watched (Speidel, 1947*b*, 1948, 1949).

Answers were sought to several questions. The first of these deals with the regenerative capacity of denervated organs. (1) Can nerveless lateral-line organs regenerate, grow, and become mature? Denervation experiments to test this have been performed on both frog tadpoles and salamander larvae. Combined [illegible]

[illegible] these organs that function as the mother-organs for the new ones that regenerate when the single operation of partial tail amputation is done. In the case of animals subjected to the combined operations, individual histories from day to day were recorded (Speidel, 1947*b*). From these it was certain that the above question could be answered in the affirmative. That is, the lateral-line sense organs were able to regenerate, grow, and become mature with the development of sense hairs and sense pores in the absence of the lateral-line nerve. It was concluded, therefore, that the

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presence of the specific nerve supply of a special sense organ is unnecessary for its origin, growth, maturation, and regeneration.

A second question deals with the problem of the maintenance of denervated organs: (2) Can nerveless organs of the lateral line persist indefinitely without structural deterioration? Long-continued case histories were necessary to settle this question. Successful observations on the same tadpoles and individual organs were made over periods of from 3 to 21 months (Speidel, 1948). The organs of a region were kept denervated by successive nerve transections, as necessary. Significant changes in the structure of surviving nerveless organs ordinarily became conspicuous after about 3–6 months. Occasionally, a nerveless organ persisted intact for more than a year. Characteristic changes of atrophy (reduction in size), dedifferentiation (loss of some features of specialized organization), and degeneration (death of the component cells of the organs) ensued.

A good comparison of nerveless and normally innervated organs was possible in the same animal in each of the experimental tadpoles. In each case, during the same period that the nerveless organs were exhibiting regressive changes, the normally innervated ones were growing and multiplying. It was concluded, therefore, that the second question could be answered in the negative. That is, nerveless organs could not maintain their structural integrity indefinitely. In other words, the specific sensory nerve supply is essential for the long-range health and survival of the special sense organs.

A third question deals with the regeneration, growth, and maturing of organless nerves: (3) Can the lateral-line nerves regenerate, grow, and become mature (myelinated) without reaching and innervating lateral-line organs? Information on this question was obtained from a number of tadpoles in which stray lateral-line nerve branches were observed (Speidel, 1948). Such aberrant branches were noted frequently in animals in which sizable gaps had been cut in the dorsal fin and also in animals following amputation of a large portion of the tail.

These cases clearly revealed that lateral-line nerve fibers could regenerate and grow long distances into new zones of tail regeneration which were devoid of lateral-line organs. Furthermore, such organless nerve fibers could mature and develop typical myelin sheath segments. The third question listed above, therefore, could be answered in the affirmative. In other words, the specific lateral-line nerve fibers could regenerate, grow, and become ensheathed with myelin, even though they were unsuccessful in innervating lateral-line organs.

The next question relates to the matter of nerve maintenance: (4) Can mature, organless, lateral-line nerve fibers persist indefinitely without structural deterioration? Prolonged observations of individual aberrant fibers covering more than 3 months were necessary to settle this point (Speidel, 1948). In two cases the observations were continued for more than a year. The aberrant fibers or, rather, aberrant distal portions of fibers were induced in some tadpoles after removal of rather large portions of the dorsal fin or of the tail tip. Some of these fibers became myelinated.

Regressive changes were noticeable, however, in all organless fibers or portions of fibers after a few months. There occurred, first, a thinning of the myelin sheath and a reduction in the caliber of the nerve fiber. Later there was complete loss of myelin. In some fibers the regressive changes did not seem to continue beyond this stage. In others the nerve substance itself finally degenerated. The sheath cells, thus stranded on an aneural length of what had been a nerve fiber, soon abandoned it either through migration and transference to adjacent live nerve [illegible]

[illegible] membrane. Ultimately, this also underwent either partial or total resorption. The fourth question above, therefore, could be [illegible]
persist in [illegible]

A final [illegible] special sensory nerves to induce the differentiation of sense organs: (5) Can the specific nerve fibers of the lateral line induce the [illegible]

[illegible] (cf. Speidel, 1948).

Following operations of tail amputation and of [illegible]

[illegible] nerve fibers in close relation to young, developing epithelium of the [illegible]

of [illegible]
the [illegible]
[illegible] that appeared in the newly regenerating terrain originated from [illegible]

rapid migration and cell division. These observations point strongly to a negative answer to the question above. In other words, lateral-line organs are not induced to form from an indifferent cutaneous epithelium through the influence of lateral-line nerve fibers; rather, they arise from pre-existing organ cells, though these may be in a dedifferentiated state.

This brief account of peripheral nerve adjustments, as observed *in vivo*, shows how cells and tissues may influence one another. The pioneer nerve growth cones are influenced by the organization of the terrain in which they advance. The later growth cones are influenced by the pioneer nerve lines. The sheath cells display an affinity for nerve fibers, and they exert a marked trophic effect on them. Nerve fibers also exert a marked trophic effect on sheath cells. Sheath cells exhibit a differential preference for myelin-emergent fibers. The sensory fibers of the vagus nerves exert a trophic influence on the special sense organs of the lateral line which they supply. Conversely, the sense organs exert a trophic influence on their innervating vagus fibers.

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NERVE REGENERATION

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BEFORE bringing some of the focal problems of nerve regeneration into the discussion, I want to state that, as to the conference itself, it seemed to me to be a great success. It was excellent and extremely interesting, and I hope that it will be followed by other conferences on the same scale. These discussions are far more important than the large, overcrowded congresses, where everyone has only ten minutes to speak and nobody is really listening to what is being said. Here you knew that only men were present who were keenly interested in the problems discussed, who knew the literature and the trend of the research work on the subjects under discussion; and the discussion itself was good and very stimulating. The films shown were extremely interesting.

The research work and the problems discussed, covering almost the entire field of the development and the regeneration of the nervous system, were interesting and of wide scope. What struck me most was that the trend of the research work and of the themes that the members of the conference were working on has altered in the course of the years. What is more or less neglected is the finer histology of the developmental problems studied. The scientists all took for granted the neuron theory (as a doctrine) and the free growing-out of the nerve fibers. The harmonious collaboration of the different elements which I tried to demonstrate in my various papers is entirely neglected, but my demonstration of the degeneration only of the conducting element after the cutting of the nerves and of the intra-protoplasmic position of the growing nerve fibers during regeneration remained unchallenged and without contradiction. Has the old controversy between neuronists and antineuronists really disappeared? Indeed, what we want is a new slogan!

One of the focal problems of the regeneration process after the cutting of a nerve is, without doubt, the question of what is degenerating—the entire axon cut off from its trophic center, the nerve cell, or only the conducting element? Second, what are the bands of Buengner, are they of lemmoblastic origin, or does the neuroplasm

of the axis cylinder, too, enter into their formation? Third, is there a free outgrowth of the axons in the scar tissue or hodogenesis in a guiding tissue of nonnervous origin; and is it possible that the outgrowing nerve sprouts are conducted by neurotropism toward the peripheral stump, with free-growing nerve fibers? Do the interstitial cells of Cajal and Lawrentjew play a role even in the regeneration process, and, if so, where do they come from? Is the lemmoblast, according to the neuron theory, everywhere to be distinguished absolutely from the real neuroblasts, the nerve cells, or is there a possible interrelation in a developmental and in a functional sense? The experiments of Professor Speidel seem to point to the first conclusion; but the films shown by him, excellent as they are, *are not absolutely convincing* because the finest histological details are not distinguishable in living substance, notwithstanding the spectacular technic and power of observation.

Let us consider the first question, What is degenerating in the nerve cell with its processes after the cutting of a nerve and the *severing of the axons from their trophic center?* As Levi has pointed *out, in the cultures in vitro* when the axons of the growing neuroblasts are severed from the perikaryon, the cut-off peripheral stump *does not* die but in many cases *even continues* to grow and in some cases fuses again with the growing central stump and lives on. It is only the conducting apparatus, the neurofibrillae, which disappears. After the section of a motor nerve the motor end-plate does not disappear, but only the conducting apparatus disintegrates and is lost, while the sarcoplasm of the end-plate (the "teloplasm," according to Noël) not only remains intact but increases in size, and the nuclei accumulate. In a degenerated end-plate I could often count more than 30 nuclei (36–39), and the same thing is seen in the beautiful preparations which Noël showed me. The mit[illegible] plasm (Noël) disappear, but [illegible] in it the multiplication of the [illegible] The same is true for the sensory corpuscles, in which the large amount of protoplasm makes it possible to study its behavior after the section of the sensory nerves.

When the degeneration of these peripheral nerve [illegible] are large [illegible] Grandry [illegible] sible, we [illegible] represents the end-ramification of the sensory nerve fiber, does not perish immediately after the section of the nerve but that it is only its neurofibrillar apparatus

which disappears and that the protoplasmic part of the disk remains visible and is used again by the regenerating neurofibrillae when regeneration has set in. Only after a time does this protoplasmic part of the tactile disk atrophy and disappear entirely when regeneration is delayed or does not occur, just as taste buds disappear when the nerves innervating them are sectioned and no regeneration sets in.

During its initial phase, that is, before a total loss of the nervous substance sets in, the degeneration process affects only the conducting mechanism—the neurofibrillar apparatus. Thus we can understand that in tissue cultures the axis-cylinder process of a neuroblast, when severed microsurgically from its trophic cell center, may remain alive and may fuse again with the new outgrowing cell process of the same cell or of another neuroblast, as in the case of Levi mentioned above. We must always bear in mind that the neurofibrillar apparatus of a nerve fiber, however dark it may be stained or impregnated, does not represent the whole nerve fiber but that it is always surrounded by a thin layer of neuroplasm. In Cajal or Bielschowsky preparations, the latter will often shrink almost to invisibility, but it remains present nevertheless. These substances together build up the nerve fiber.

That the cut-off axon is changing its aspect as a whole is undeniable. Not only the myelin sheath and the cells of Schwann undergo many changes, as Cajal has described and drawn so beautifully, but even the axon itself suffers deteriorating changes. But, after all, it is chiefly the neurofibrillar apparatus which degenerates, alters its chemical composition, and disintegrates and finally disappears entirely. Even in the "digestive chambers" of Cajal it is only a part of the neurofibrillar apparatus which is digested, and the entire axon may pass a digestive chamber without interruption. Indeed, when studying the degeneration process as carefully as possible, I have always got the impression that the neuroplasm of the axon changes its aspect in connection with the decomposition of the neurofibrillar apparatus and of the surrounding sheath substance but that it survives partly and fuses with the protoplasm of the surrounding elements (cells of Schwann). The decomposition substances are eliminated and disappear, the nuclei multiply by mitosis, and out of this fusion of the protoplasmic substances the protoplasmic bands of Buengner are built up. In regeneration, the outgrowing neurofibrillar apparatus follows these conducting protoplasmic bands inside the neurilemmal sheath as fine differentiations lying inside this protoplasm, as was described by Cajal himself. But the outgrowing nerve fibers, the

axons, one may ask, do they not grow through the scar tissue as free fibers with terminal clubs between the other elements of the scar until they meet the conducting peripheral tubes with the bands of Buengner, as was described and drawn so beautifully by Cajal?

This is one of the most difficult questions to answer because in the Cajal and Bielschowsky preparations the neuroplasm of the thin nerve fibers often shrinks almost to invisibility, so that only the black streaks of the neurofibrillar apparatus are to be seen. But when we fix the pieces as carefully as possible and study them with the highest magnification and strong light, after having stained them with a protoplasm counterstain, we see not only that these black threads are enveloped everywhere by a thin layer of protoplasm but that real nuclei are present in this layer, accompanying the growing nerve fibers. According to Cajal (Cajal, *Regeneration of the Nervous System* [1913], in the translation by May, 1928, p. 168), all the *nerve sprouts* that circulate through the scar during the first week are naked fibers in which not only the myelin but the cells of Schwann are lacking. No satellite nuclei are present. But when we see the drawings by Cajal himself, as, for instance, the drawing of the beginning of outgrowth of the axons from the central stump (Cajal [1908], p. 33) which I reproduced in my paper on nerve regeneration in Bumke-Foerster's *Textbook of Neurology* (1937), Part I, page 1036, or the drawings in his book on regeneration (Figs. 68, 69, 70, and others), they cannot, with the best will in the world, be interpreted otherwise than as proving that the outgrowing fibers from the very beginning are surrounded by a protoplasmic sheath with nuclei accompanying them and that they do not appear as free-growing naked fibers. Thus is what my own preparations have taught me.

To make my meaning clear, I would emphasize that I am not attacking the usual description of the nerve-regeneration process as it has been studied by the most illustrious histologists of our times, but I am only trying to show that the fourth point set forth by Cajal as one of the arguments of the neuron theory—viz., that every neuron together with all its processes, including the axon, has developed from a single cell or neuroblast without any co-operation of other nervous or neurological elements and that, after section of the axon, the cell body of a neuron causes the regenerative outgrowth of the new axon without any collaboration of other protoplasmic elements—is not so indubitably and securely settled as is assumed in most of the treatises and textbooks which deal with this question. There are, even here, many problems of fundamental importance still unanswered. The

details of the regeneration process, when outgrowing nerve fibers are studied in preparations in which the surrounding elements and the protoplasm also are stained, point far more strongly to a harmonious co-operation of all the elements necessary and, indeed, indispensable to the final result—the restoration of the harmonious function of the end-formations—than to a simple process of outgrowth of regenerating free nerve fibers running between the surrounding elements without any force to guide them but the highly hypothetical neurotropic influence of the distal nerve stump.

Is there a neurotropic influence to guide the outgrowing nerve fibers during regeneration toward the peripheral stump, with free-growing nerve fibers, or is it a hodogenetic process with a conducting tissue of nonnervous origin? Do the interstitial cells of Cajal and Lawrentjew play a role even in the regeneration process, and, if so, where do they come from? In the often very dense tissue of the scar, with its often inextricable tangle of growing and pushing and dividing nerve sprouts, it is impossible to give a definite answer to these questions. We have to look for a more loosely built tissue in which the regenerating nerve sprouts may be studied better in their relations to the surrounding elements. Such a tissue is found in the muscle spindles, which I studied years ago in the cat, and especially in the hedgehog, with its large cellular elements. In the lymph space surrounding the muscle fibers of the spindle, cells are very sparse, as is the case in the loose connective tissue of the iris of monkeys and man, and the relation of the regenerating nerve fibers to their surroundings is very clear. During the first weeks after the cutting of the nerves, the only change in these cells, which anastomose with one another as true connective-tissue elements, is that they proliferate, send out new processes, building a denser tissue, as has already been demonstrated by Tello. According to Tello and Cajal, the growing nerve fibers, having penetrated the lamellated connective-tissue sheath and having arrived inside the lymphatic periaxial space, bridged by the connective-tissue cells mentioned above, proceed as absolutely free fibers until they reach the central muscle fibers of the spindle ("aussitôt qu'elles ont pénétré, elles perdent leur enveloppe et ainsi libres entre les fibres musculaires, elles commencent à former leur ramification terminale"; and, a little later, "nulle part dans le trajet des fibres après la disparition de leur enveloppe nous n'avons pu trouver les cellules marginales qui, d'après les partisans de la théorie caténaire, seraient nécessaires, etc." [*Tello (1907),*

pp. 8 and 9]). With this description I cannot concur. I formulated the results of a careful investigation of longitudinal and transverse sections stained with silver and afterward treated with chloride of gold and thionin as follows: "There are in the neuromuscular spindles no free 'naked' fibers, neither in the normal spindles nor during regeneration after the cutting of the nerves. Every nerve fiber lies imbedded in its sheath of protoplasmic cells, seemingly of connective-tissue origin." I can maintain this conclusion at every point, even at present. The elements of the lymphatic space, in which the [illegible]

[illegible]

That growing nerve fibers may proceed entirely free, without the collaboration of conducting elements, has been demonstrated beyond doubt by Harrison, by Cajal, by Burrows and Lewis, and afterward by Hooker and Speidel in their extremely interesting experiments. But then these nerve fibers grow out in a straight line without distinct alteration of course. But when they have to follow a definite functionally important course, as in regeneration inside the body, they need a conducting tissue, which regulates *their course*, which constitutes a "hodogenetic" path, as was pointed out so eloquently by Dustin many years ago As soon as the original peripheral nerve stump ("tubes orientateurs" of Cajal) is reached, the outgrowing nerve fibers find *there a conducting course, and they grow* farther out inside the protoplasmic sheath of the bands of Buengner until they reach their final goal, the terminal region. In this terminal region we see, in the first place, a proliferation of the Schwann elements, by which proliferation the conducting tissue is developed and elongated, which *brings* the outgrowing nerve fibers in even closer contact with the terminal elements. But they need another terminal conducting tissue to establish the final contact with the elements to be re-innervated. According to Cajal and Tello, the ends of the "tubes orientateurs," the Sch[illegible] *ing fibers* pass out of t[illegible] they reach their final [illegible] muscle fiber or the sensory end-corpuscle, toward which they are attracted by neurotropism. In the muscle spindles, where the conditions are very favorable, it is easy to see that the fibers are not running freely between the cellular elements, that the ends of the Schwann cells, *of* the "tubes orientateurs," *are not* open, but that *the tubes* are elongated into a protoplasmic conducting tissue composed of anastomosing elements of

details of the regeneration process, when outgrowing nerve fibers are studied in preparations in which the surrounding elements and the protoplasm also are stained, point far more strongly to a harmonious *co-operation of all the elements necessary and, indeed, indispensable* to the final result—the restoration of the harmonious function of the end-formations—than to a simple process of outgrowth of regenerating free nerve fibers running between the surrounding elements without any force to guide them but the highly hypothetical neurotropic influence of the distal nerve stump.

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tive function or as a mode of defense against the surrounding milieu or any other function, is a fact not to be denied. There is no separating membrane between, but only *living* substance, as in the synaptic junctions. A surface of separation between the two protoplasms is apparent, but there is no intermediate inert material, as the old classic neuron theory maintained. In regeneration, "hodogenesis," a contact with living substance, is necessary. The nervous elements may be independent, but they are not isolated in the living organism. There must be a living contact between them and the surrounding living elements.

In the protoplasmic bands of Buengner, the neurofibrillae and their neuroplasm lie imbedded in the protoplasm of the bands. That there must be a surface of separation between the two protoplasms is obvious, and the growing nerve fibers, even when imbedded in this protoplasmic band, retain their physiological independence. But even when the protoplasmic bands of Buengner become vacuolated when the myelin sheath is formed and the bands of Buengner change into a myelinated nerve fiber (or into a number of such fibers), the intra-protoplasmic position of the neurofibrillae remains obvious and is clearly visible, especially in cross-sections, the only kind giving conclusive results. This seems to me to point to a composition of these protoplasmic bands of sheath cell protoplasm and of neuroplasm from the axons, just as could be deduced from the origin of the bands, as mentioned above. Is there a *fusion* of the two living substances? In the normal motor end-plate the neuroplasm of the motor nerve fiber fuses with the sarcoplasm of the sole plate, constituting a functional unit. There is no separating membrane, not even a discernible interface. A separate functional unit of the motor end-plate is formed in development after an earlier syncytial stage of the nerve ends. From this syncytial stage arise the separate motor end-plates. The cell lineage of the adult elements is often by no means clear. In this light it is not surprising that, among the different parts of the body, it is the nervous system which must primarily exhibit phenomena in harmony with this view, in which this higher "functional unit" plays a more dominant part. A plexiform motor innervation, forming a functional unit beyond the usual cellular unit of life throughout life by retaining the original developmental syncytial stage, is found, for instance, in the cross-striated ciliary muscle of the bird's eye (Crampton's muscle). But a more extensive discussion of these questions lies beyond the scope of the present article.

apparently mesenchymal origin, in which the nerve fibers lie intra-protoplasmically (in cross-sections often seen lying in an indentation of the nucleus, which makes their intra-protoplasmic position undeniable). It is difficult to tell the exact origin of the anastomosing elements in the protoplasm of which the nerve fibers are imbedded after having left the bands of Buengner and the "tubes orientateurs" (Schwann sheaths), but they are everywhere anastomosing with the stellate cells lying in the lymphatic space surrounding the muscle fibers of the spindle, and they form a cellular sheath in which the neurofibrillar strands are imbedded intra-protoplasmically. This can be clearly seen, and as a histological fact it is, in my opinion, undeniable; but, although their form (stellate and anastomosing through protoplasmic processes, with other mesenchymal connective-tissue cells lying inside the lymphatic space of the muscle spindle) points to a mesenchymal origin, their origin remains unsettled, in need of other than purely histological investigations. I shall return to this problem and to the interstitial cells later on, but I want here to quote Cajal himself, the past master of the histology of the nervous system. In his book on the development and the regeneration of the nerve fibers, Cajal, who could not deny the intra-protoplasmic position of the regenerating fibers in the surrounding elements, describes this as a sort of symbiosis, in which the surrounding cells act as receptacles. According to Cajal, these elements are of mesenchymal origin, and he acknowledges the possibility that they may subserve a nutritive function. Considering, then, that Tello (*Trabajos Madrid* [1911]), in the course of his discussion of neurotropism in regeneration in the central nervous system, mentions a newly formed connective tissue that may act as a conductive tissue for the regenerating nerve fibers and that de Castro (1933) writes "même en admettant la pénétration intraprotoplasmique d'une neurofibrille dans une cellule d'origine mésenchymateuse, il n'est pas moins certain que la fibre terminale et le protoplasma innervé continuent à rester indépendants puisque les protoplasmas ne se confondent pas," it seems to me that even in the camp of the most convinced neuronists there is room for the views expressed here. That the functional independence of the imbedded nerve fibers remains absolutely intact, I need not stress here. The physiological independence of the neurons is a definite and almost undisputed reality, but we must be careful not to confuse this physiological independence with an anatomical independence in the sense of the old neuron theory. The collaboration of elements of different origin with nervous elements, be it in a nutri-

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apparently mesenchymal origin, in which the nerve fibers lie intra-protoplasmically (in cross-sections often seen lying in an indentation of the nucleus, which makes their intra-protoplasmic position undeniable). It is difficult to tell the exact origin of the anastomosing elements in the protoplasm of which the nerve fibers are imbedded *after having left the bands of Buengner and the "tubes orientateurs"* (Schwann sheaths), but they are everywhere anastomosing with the stellate cells lying in the lymphatic space surrounding the muscle fibers of the spindle, and they form a cellular sheath in which the neurofibrillar strands are imbedded intra-protoplasmically. This can be clearly seen, and as a histological fact it is, in my opinion, undeniable; but, although their form (stellate and anastomosing through protoplasmic processes, with other mesenchymal connective-tissue cells lying inside the lymphatic space of the muscle spindle) points to a mesenchymal origin, their origin remains unsettled, in need of other than purely histological investigations. I shall return to this problem and to the interstitial cells later on, but I want here to quote Cajal himself, the past master of the histology of the nervous system. In his book on the development and the regeneration of the nerve fibers, Cajal, who could not deny the intra-protoplasmic position of the regenerating fibers in the surrounding elements, describes this as a sort of symbiosis, in which the surrounding cells act as receptacles. According to Cajal, these elements are of mesenchymal origin, and he acknowledges the possibility that they may subserve a nutritive function. Considering, then, that Tello (*Trabajos Madrid* [1911]), in the course of his discussion of neurotropism in regeneration in the central nervous system, mentions a newly formed connective tissue that may act as a conductive tissue for the regenerating nerve fibers and that de Castro (1933) writes "même en admettant la pénétration intraprotoplasmique d'une neurofibrille dans une cellule d'origine mésenchymateuse, il n'est pas moins certain que la fibre terminale et le protoplasma innervé continuent à rester indépendants puisque les protoplasmas ne se confondent pas," it seems to me that even in the camp of the most convinced neuronists there is room for the views expressed here. That the functional independence of the imbedded nerve fibers remains absolutely intact, I need not stress here. *The physiological independence of the neurons* is a definite and almost undisputed reality, but we must be careful not to confuse this physiological independence with an anatomical independence in the sense of the old neuron theory. The collaboration of elements of different origin with nervous elements, be it in a nutri-

tive function or as a mode of defense against the surrounding milieu or any other function, is a fact not to be denied. There is no separating membrane between, but only *living* substance, as in the synaptic junctions. A surface of separation between the two protoplasms is apparent, but there is no intermediate inert material, as the old classic neuron theory maintained. In regeneration, "hodogenesis," a contact with living substance, is necessary. The nervous elements may be independent, but they are not isolated in the living organism. There must be a living contact between them and the surrounding living elements.

In the protoplasmic bands of Buengner, the neurofibrillae and their neuroplasm lie imbedded in the protoplasm of the bands. That there must be a surface of separation between the two protoplasms is *obvious*, and the growing nerve fibers, even when imbedded in this protoplasmic band, retain their physiological independence. But even when the protoplasmic bands of Buengner become vacuolated when the myelin sheath is formed and the bands of Buengner change into a myelinated nerve fiber (or into a number of such fibers), the intra-protoplasmic position of the neurofibrillae remains obvious and *is clearly visible*, especially in cross-sections, the only kind giving conclusive results. This seems to me to point to a composition of these protoplasmic bands of sheath cell protoplasm and of neuroplasm from the axons, *just as could be deduced* from the origin of the bands, as mentioned above. Is there a fusion of the two living substances? In the normal *motor end-plate* the neuroplasm of the motor nerve fiber *fuses* with the sarcoplasm of the sole plate, constituting a functional unit. There is no *separating* membrane, not even a discernible interface. A separate functional unit of the motor end-plate is formed in development after an *earlier syncytial* stage of the nerve ends. From this syncytial stage arise the separate motor end-plates. The cell lineage of the adult elements *is often* by no means clear. In this light it is not surprising that, among the different parts of the body, it is the nervous system which must primarily exhibit phenomena in harmony with this view, *in which* this higher "function[illegible]" plays a mor[illegible] ing a functi[illegible] life by retai[illegible] [illegible]opmental syncytial stage, is found, for instance, in the cross-striated *ciliary* muscle of the bird's eye (Crampton's muscle). But a more extensive discussion of these questions lies beyond the scope of the present article.

Two points still need to be considered here—(1) in view of the syncytial stage in the development and regeneration of the terminal nervous apparatus, the question of degeneration in a reticulate nervous structure, as, for instance, the sympathetic ground plexus, and (2) the question of whether the cells of apparently mesenchymal origin in the muscle spindle, that form a guiding structure for the regenerating nerve fibers, may be regarded as "interstitial cells" or not. In discussing these two points I may follow the discussion in my book on *Problems of Nervous Anatomy* (Oxford, 1940).

1. In the sympathetic ground plexus, which is present throughout the whole body, following the blood vessels and the muscle fibers, running in the connective tissue, innervating the different glands, the fat cells, etc., the existence of a real netlike structure of the neurofibrillar apparatus is indubitable, quite apart from any artificial aspects caused by the fixing agent. Even in sections in which the thicker neurofibrillar strands are seen as nonvaricose smooth threads running seemingly independently of one another (for instance, in the coats covering the eyeball in tangential sections) closer examination reveals exceedingly delicate anastomosing varicose fibers of this ground plexus with astonishing clearness, which leaves no doubt about their forming a real netlike structure. In methylene blue preparations the same reticulate sympathetic nervous plexus is plainly visible (Leeuwe). Histologically, a reticular structure of the sympathetic ground plexus seems to be beyond doubt. Now one may ask whether the results of the degeneration experiments of Lawrentjew, Kolossow, and Schimert (1937, 1938), who found degenerating nerve strands in the sympathetic plexus of the wall of blood vessels after cutting some of the sympathetic nerves, do not (as they maintained) directly contradict the possibility of the existence of a netlike structure of the ground plexus. On the basis of their experiments the authors themselves positively deny the existence of a reticular sympathetic plexus. But, first, in their drawings the droplets of the degenerating axis cylinders are seen chiefly in the thicker nerve bundles, where neurofibrillae may still be running separately, and, second, what do we know of the conducting process of the nervous stimulus and about the course of the degenerative process in and through a reticular plexus in which the different neurofibrillar strands are so obviously anastomosing with one another? We do not yet know anything about the course of the degenerating process in the loose reticular strands of the sympathetic ground plexus or about its course in the sensory ground net, which Stefanelli demonstrated in

the connective tissue of the skin, or in the sympathetic reticulum of Ceccherelli. Thus the results of the degeneration experiments mentioned above do not seem to give a conclusive answer to the question of the reticular structure of the ground plexus. It still remains for the future to study more accurately the degeneration and regeneration in a reticular nervous structure.

2. Are the cells of apparently mesenchymal origin, forming a conducting system for the regenerating nerve fibers, to be regarded as "interstitial cells" in the sense of Cajal, Lawrentjew, and Leeuwe? Is there a connection possible between the terminal nerve fibers and elements of a mesenchymal nature, especially in the case of the regeneration of terminal nerve fibers, in which a conducting structure for them is necessary? The connection of sympathetic nonmedullated nerve fibers to elements of mesenchymal origin is an exceedingly difficult question to resolve. Goormaghtigh pointed out (1924) the "close bonds of relationship which unite the ganglion cells of the spinal ganglia, the Schwann-cells and the chromaffin cells in the adrenal gland." Are there sympathicoblasts there? Masson and Berger showed (1923) that the same sort of relationship exists between the sheaths of the nonmedullated nerve plexuses and the interstitial cells of the ovary and of the testicle, which are also of mesenchymal origin. The origin of the sympathicoblasts in general is still by no means clear, and even at the present time an origin from mesenchymal elements is maintained.

In the pancreas, van Campenhout (1925, 1927) has studied the intimate connections which certain cells of the primary islands of Langerhans (Laguesse) make with the plexus of sympathetic nerves. These relations concern only the satellite apparatus. In the cornea, Nageotte and Guyon (1926) described a [illegible] superficial, but even he agrees that "these nerve fibers sometimes when in contact with a cell throw out extremely fine collaterals which follow one border of the cell"; and he adds: "Some writers *speak* of an innervation of connective cells, and Guyon and I have made observations that are not unfavorable to this interpretation" (1931). *In* the frog and in birds and, according to Stefanelli (1938), also *in reptiles*, this connection is so apparent as to leave no doubt as to its reality. In the human cornea, I could show that the very delicate varicose neurofibrillar strands run in the protoplasm of stellate cells, anastomosing with one an-

other and being inclosed in the connective-tissue network. In several cases the fine neurofibrillar thread was lying in a narrow groove of the nuclear membrane, with a varicosity at both ends of the nuclear groove. This shows not only that the position of the neurofibrillar strands is superficial, so that they "*follow one border of the connective-tissue cells,*" as Nageotte described, but that they are really intra-protoplasmic and intra-cellular. I never found thin *neurofibrillar strands in the connective tissue of the cornea lying free between the cells.*

In the iris of mammals (monkeys), there exists such a close connection between the sympathetic nonmedullated nervous plexus and the apparent connective-tissue cells that, even as long ago as 1905 and 1910, these connective-tissue cells were described as true interstitial cells of Cajal (Muench, 1905; Schock, 1910; Wolfrum, 1931). In the iris of monkeys and man we find the most interesting loose connective tissue that I have ever seen. It contains, at least in the so-called "vascular" layer, numerous blood vessels, the spaces between which are filled with such a loose, spongelike connective tissue *that at first sight in ordinary preparations only the black or brown* branched chromatophores connected by thin spider-like fibroblasts are seen extending with long branches in an apparently fluid-filled space. *Only when the connective tissues are stained very carefully* do we see that this space is filled with very loosely arranged, wavy, collagenous fibrillae. In this loose connective tissue, nerve fibers run everywhere, and even the thin nerve fibers are very loosely arranged. They form not only a plexus but a real network, as can be recognized beyond doubt in silver preparations and after staining with methylene blue. This network of neurofibrillar strands is in such close connection everywhere with the cells mentioned above that one can only speak of a syncytial arrangement in which the neurofibrillae run inside the protoplasm of the cells. This syncytial arrangement is so evident that, as I mentioned above, even as long ago as 1905 and 1910 all these cells were identified with the interstitial cells of Cajal, and it was maintained that all the branched cells in this tissue were of nervous origin and nothing but interstitial cells and that every chromatophore was innervated by them. Expressed thus, this statement is incorrect, but it contains a nucleus of truth. There is a close syncytial protoplasmic connection between the finest neurofibrillae and the *surrounding elements, as in the cornea described above. In* this way these elements are to be regarded as true interstitial cells. In carefully made preparations through an iris of a newborn ma-

caque, in which the chromatophores are not yet black but only of a greenish-brown transparent color, it was even possible to state that the chromatophores are innervated by the same delicate neurofibrillae. However, not every branched cell of the connective-tissue stroma of the iris can be considered an interstitial cell.

We find in the iris true ganglion cells, with large, round nuclei and numerous Nissl bodies in their protoplasm These ganglion cells lie in groups in the loose connective tissue and are connected with the nervous strands. In silver preparations and in preparations stained with methylene blue we find small multipolar cells with a network of neurofibrillae inside their protoplasm in connection with the neurofibrillar strands of the nervous network mentioned above. They lie in a syncytial arrangement and in distinct protoplasmic connection with the branched cells of the connective tissue of the stroma of the iris. Leeuwe (1937) was able to demonstrate that Nissl bodies are present in their cytoplasm, which thus proves their nervous nature. These elements are true interstitial cells of Cajal. Here in the loose connective tissue of the iris they can be studied with the utmost accuracy, and they appear as small ganglion cells forming a *syncytium*, connected and anastomosing everywhere with the cells of connective-tissue origin mentioned above.

Whether one may speak here of an "innervation of the connective tissue cells" as Nageotte puts it for the elements of the *cornea*, is difficult to say, but in my opinion it demonstrates the peculiar character of these interstitial cells as intermediate elements of a nervous nature in protoplasmic connection with elements of a true connective-tissue origin and character. They innervate the muscle elements of the iris, and, together with the sympathetic plexus in which they lie, they must be assumed to be efferent in nature.

But are they of nervous *origin*? They contain true neurofibrillae and are in syncytial connection with true mesenchymal elements. Is it not possible that they are of true mesenchymal origin but transformed into interstitial intermediate elements of a nervous, but intermediate, function? We may refer to the fate of mesectoderm in development, to the sympathicoblasts, to the transformation of endothelial cells into histiocytes, of fibroblasts into smooth muscle cells and even into fat cells—*in short*, to the widespread capacity of the elements of the organism to transform. In the experimental embryological field we know of the forming of a lens from the iris epithelium, of a true lens out of the brain elements, of mesenchymal renal tubes, etc., out of ectodermal elements, in short, everywhere

we see the possibility *of transformation, in order to maintain the* harmony of the body, of the different parts of the organism. Why not in the case of the interstitial elements?

Here I must ask forgiveness for going too far into purely histological details and problems, but it was necessary in order to procure a sound basis for the discussion of the problem of the role played by what appear *to be connective-tissue cells in the lymphatic space* surrounding the thin muscle fibers of the muscle spindle, which elements played such a prominent part in conducting the regenerating nervous strands to their goal, the innervation of the muscle elements of the spindle. There, too, the regenerating nerve fibers are inclosed in the protoplasm of conducting elements, true stellate, anastomosing cells of mesenchymal nature and behavior, anastomosing with true connective-tissue cells. *This is a fact, not a mere pretention. Cajal speaks* in such a case of "symbiosis," but in my opinion this is merely a play upon words; as I have already said, the neuroplasm surrounding the neurofibrillar strands lying in the protoplasm of the conducting cell is and remains different from the cytoplasm of the conducting cell, but it is a connection of living material, of living substance, without a separating membrane or, even less so, inert material between the two *individual elements; and there must be an integrative* action between them, be it of a nutritive character or exciting or regulating—in short, an integrative action as we see it in the whole harmonious organism, and especially in the nervous system.

Seen in this light, the problem of the co-operation of the different elements in the regeneration, as in the development, of the nervous system, the problem of the interstitial cells as an intermediate structure and the *problem of the conducting elements, be they of neurogenic or of mesenchymal origin,* is one of the focal problems of the process of nervous regeneration. It lies at the base of our conception of regeneration as one of the harmonious processes to maintain the homoeostasis of the living organism. When we regard the nervous system as an isolated system, according to the neuron theory in its classic form, we are on the wrong track.

Perhaps I may be allowed to finish this short article,[1] meant only to express my sincere thanks for the excellent discussion we enjoyed so much during the conference in Chicago, with a reference to what I

[1] The problems under discussion here are treated more elaborately in my paper on the synaphology, etc., of the sympathetic end-formation in *Acta anatomica* (Basel), VIII (1949), 18–61.

said some years ago: Histology, especially in the study of nervous regeneration, is interesting only when it gives a better insight into the function of the tissue or elements under discussion. Theories based upon principles once commonly accepted but now obsolete are likely to lead to an uncompromising attitude which kills progress. This is the case with the classical neuron theory. The words of Claude Bernard may well be quoted here: "Il faut chercher à briser les entraves des systèmes philosophiques et scientifiques, comme on briserait les chaînes d'un esclavage intellectuel." This progressive outlook does not, of course, in any way detract from the historical value of such theories or from the genius of those who formulated them. The question of the regeneration of the nervous system has to be studied morphologically and physiologically, but in this study it is dangerous to begin with the classical neuron theory as its unshakable basis, for this would lead us further and further away from the ascertained facts and hamper us in every respect, both histologically and physiologically. It is always dangerous to label a scientist as "neuronist" or as "antineuronist" or "reticularist." We have simply to try to understand rightly the complicated structure of the nervous system, its plasticity and, on the other hand, its conservativeness and its integrative action. We do not need to adhere to obsolete conceptions and formulae of the last century. We need to form new paths, to achieve new outlooks, to admit that the structure of the body is far more complicated than the older scientists thought it to be. But in this we must not be impeded and obstructed by obsolete formulae, however important they may have been for the advancement of science in the past and however important they remain. We have to search for a new basis or more modern structure than the old classical neuron theory. As Cajal himself said in his last paper (1936): "We must never forget that our knowledge of the nervous system is still very incomplete and that the leading thought of the histologist must be a wise skepticism. A science which acquired its methods only forty years ago cannot already possess the key for the understanding of its structure, which will be the greatest problem for years to come."

THE DETERMINATION OF THE SPECIFIC CHARACTERISTICS OF NERVE FIBERS

J. Z. YOUNG

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EVIDENCE that nerve fibers possess specific differences has been accumulating for a long time and was repeatedly mentioned during the Chicago symposium. No attempt will be made here to survey these differences exhaustively, but it may be useful to list some of them and to consider the evidence available about the factors that have led to the establishment and maintenance of the differences.

The distinctions known to exist between nerve fibers of any one individual may be classified roughly as (1) structural differences, which include differences in diameter, presence or absence of a myelin sheath, and variations in its thickness and variations in internode length, and (2) chemical differences: there are known to be marked differences between nerves in the content of acetylcholine and cholinesterases and of adrenalin and similar substances. In addition, there are almost certainly many other specific chemical characteristics of particular fiber types; for example, there is something in both sympathetic and sensory nerve fibers that makes it impossible for them to establish connection with motor end-plates of somatic muscle (Simpson and Young, 1945; Gutmann, 1945; Weiss and Edds, 1945).

A certain amount of information is available about the determination of these differences between nerve fibers during development, as the originally similar neuroblasts come to acquire their adult differences; but we shall consider here the means by which these differences are maintained and reproduced during adult life and throughout the process of nerve regeneration. The fact that in the adult a newly outgrowing nerve fiber is attached to an already differentiated neuron provides us with the possibility of investigating the nature of the influences that extend over the cell, causing reciprocal effects between the cell body and its newly formed regenerative process. The specific characteristics of each nerve fiber seem to depend on a complex of influences, some emanating from the cell body,

others from the end-organ or other nerve cell with which the tip of the axon is in contact. The problem of the maintenance of the specific characteristics of the nerve fiber, therefore, involves the whole question of the nature of the "trophic influences" that proceed along the nerve cell in both directions (Young, 1946*a*).

It is not possible here to survey all the known information about the effects that one part of a nerve cell exercises on distant regions of the same cell. The propagation of the action potential is the specific means by which the adult nerve cell discharges its special function of conduction, but it may very well be that this spread of activity also plays a large part in allowing interaction between the metabolism of distant parts of the cell. Indeed, it is not improbable that the discharge which we know as the nerve action potential is the special development of a phenomenon common to the surfaces of all cells and that it is responsible for the maintenance of the integrity of cells as complex specific action systems that are in some sense discrete from *their* environment. It may be, that is to say, that the action potential has "trophic" effects which are characteristic of cells in general.

We must be careful, however, about ascribing "trophic maintenance" to the conduction of nerve impulses in the usual directions. Afferent nerve fibers severed from their end-organ probably decline in diameter (see later), thus showing some effect of the absence of their normal load of impulses, but they certainly do not show the signs of Wallerian [illegible] afferent n [illegible] in connect [illegible] periphery soon ceases to be able to conduct. Within about three days (in a mammal) it will be undergoing fragmentation [illegible] potentials.

In seeking to understand this trophic influence, we naturally look for further evidence that interchanges of material may take place along the length of a nerve fiber. Stress has often been laid on the very great disproportion between the length and the breadth of the fiber. It has been argued that it would take an astronomical length of time for substances issuing from the cell body to reach the periphery by diffusion alone In a large mammalian nerve fiber the axon is about 15 μ in diameter and may be upward of a meter long; in the smaller fibers the disproportion is still greater [illegible] considerable [illegible] place by proc [illegible]

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thers from the end-organ or other nerve cell with which the tip of he axon is in contact. The problem of the maintenance of the spe- ific characteristics of the nerve fiber, therefore, involves the whole uestion of the nature of the "trophic influences" that proceed along he nerve cell in both directions (Young, 1946*a*).

It is not possible here to survey all the known information about he effects that one part of a nerve cell exercises on distant regions of he same cell. The propagation of the action potential is the specific neans by which the adult nerve cell discharges its special function of onduction, but it may very well be that this spread of activity also lays a large part in allowing interaction between the metabolism of istant parts of the cell. Indeed, it is not improbable that the dis- harge which we know as the nerve action potential is the special de- elopment of a phenomenon common to the surfaces of all cells and hat it is responsible for the maintenance of the integrity of cells as omplex specific action systems that are in some sense discrete from heir environment. It may be, that is to say, that the action potential as "trophic" effects which are characteristic of cells in general.

We must be careful, however, about ascribing "trophic main- enance" to the conduction of nerve impulses in the usual directions. fferent nerve fibers severed from their end-organ probably decline n diameter (see later), thus showing some effect of the absence of heir normal load of impulses, but they certainly do not show the igns of Wallerian degeneration. On the other hand, a portion of an fferent nerve fiber separated from its nerve-cell body but remaining n connection with the periphery soon ceases to be able to conduct. Within about three days (in a mammal) it will be undergoing frag- nentation. Clearly, the "trophic influence" by which the peripheral arts of a fiber are normally maintained is not mediated only by the ropagated action potentials.

In seeking to understand this *trophic influence*, we naturally look or further evidence that interchanges of material may take place long the length of a nerve fiber. Stress has often been laid on the ery great disproportion between the length and the breadth of the iber. It has been argued that it would take an astronom...

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THE DETERMINATION OF THE SPECIFIC CHARACTERISTICS OF NERVE FIBERS

J. Z. YOUNG

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EVIDENCE that nerve fibers possess specific differences has been accumulating for a long time and was repeatedly mentioned during the Chicago symposium. No attempt will be made here to survey these differences exhaustively, but it may be useful to list some of them and to consider the evidence available about the factors that have led to the establishment and maintenance of the differences.

The distinctions known to exist between nerve fibers of any one individual may be classified roughly as (1) structural differences, which include differences in diameter, presence or absence of a myelin sheath, and variations in its thickness and variations in internode length, and (2) chemical differences: there are known to be marked differences between nerves in the content of acetylcholine and cholinesterases and of adrenalin and similar substances. In addition, there are almost certainly many other specific chemical characteristics of particular fiber types; for example, there is something in both sympathetic and sensory nerve fibers that makes it impossible for them to establish connection with motor end-plates of somatic muscle (Simpson and Young, 1945; Gutmann, 1945; Weiss and Edds, 1945).

A certain amount of information is available about the determination of these differences between nerve fibers during development, as the originally similar neuroblasts come to acquire their adult differences; but we shall consider here the means by which these differences are maintained and reproduced during adult life and throughout the process of nerve regeneration. The fact that in the adult a newly outgrowing nerve fiber is attached to an already differentiated neuron provides us with the possibility of investigating the nature of the influences that extend over the cell, causing reciprocal effects between the cell body and its newly formed regenerative process. The specific characteristics of each nerve fiber seem to depend on a complex of influences, some emanating from the cell body,

some liquid properties; many of the classical properties of nerve fibers can be shown to be those that would be expected of a very long, thin column of viscous liquid, inclosed in a wall that resists stretch, has some elasticity, and is permeable to some of the liquid contents. Thus the incisures of Schmidt-Lantermann have a periodicity of about twice the diameter of the fibers, which suggests that they are due to the tendency of a liquid cylinder to break into a series of stable ovoids. The nodes of Ranvier show a minimum periodicity of about 200–250 μ in a variety of animals, suggesting that the myelin adopts the figure of a very elongated droplet.

DETERMINATION OF THE DIAMETER OF NERVE FIBERS

The differences in diameter provide perhaps the most obvious of all the specific characteristics of nerve fibers. In mammals the difference in axon diameter between the largest and the smallest fiber is nearly twenty times; together with the associated differences in myelination, this produces a difference in conduction velocity of about one hundred times. It is still uncertain to what extent these differences are of functional significance, but we are now beginning to understand the factors that produce them. The whole problem can be settled only by [illegible] as those of Weiss, [illegible] ies of nerve regene[illegible] by showing the limits of the extent to which differences of nerve-cell size control axon diameter and thus emphasizing the importance of the peripheral influences acting upward along the nerve.

When nerve fibers grow out from a severed nerve stump, across a point of union, and down into a peripheral stump, they are very small at first, 1 μ in diameter or less. Whether they subsequently become large depends on two factors: (1) the size and nature of the cell body from which they originate and (2) whether they make appropriate contacts with a peripheral end-organ.

The effect of the cell body is well shown in experiments in which nerves are made to grow into unusual channels (see Simpson and Young, 1945). Thus the nonmedullated postganglionic fibers of the mesenteric nerves of the rabbit can be directed into the [illegible] stump of a cut [illegible] large neurile[illegible] but, in spite [illegible] Schwann cells, they will not grow large or acquire myelin sheaths. The sympathetic [illegible]

tive evidence about the physical condition of the axoplasm, but it certainly can behave like a liquid. Thus Causey and Palmer (1949) have recently shown that mercury or air pressures above 100 mm. Hg applied round a nerve produce reversible displacement of the axoplasm from the region compressed. Moreover, they found evidence of a slight tendency for the material to be moved distally toward the periphery rather than upward toward the nerve-cell body. Hodgkin and Katz (1949) have shown that squid axoplasm behaves as a gel but that its viscosity falls greatly when in contact with calcium ions. *It may be that during life there are considerable fluctuations in* viscosity; certainly, axoplasm shows some gel properties and can also, in life, behave like a liquid. For example, the axoplasm of the giant nerve fibers of the polychaete worm *Myxicola* is so viscous that it is difficult to insert a needle into it. Yet every time that the worm contracts, the axoplasm changes from a cylinder about 500 μ in diameter and 10 cm. long to a conical figure reaching 1500 μ in diameter and as little as 4 cm. in length (Nicol and Young, 1946; Nicol, 1948). It needs no further evidence to show that the axoplasm presents some fluid properties.

The experiments of Weiss (1943) and of Weiss and Hiscoe (1948) in which the axoplasm becomes dammed up behind a constricted region suggest that some transport along the nerve fiber occurs during life, *proceeding away from the cell body toward the periphery. We do not* know what forces produce these movements, but there is evidence from the observations of Speidel in the tadpole's tail and of Lewis and others in tissue culture that the living nerve fiber is far from being the inert, motionless structure that we imagine from fixed preparations. Granules can be seen moving for considerable distances in these living nerves, and they strongly support the suggestion that the nerve fiber has liquid properties and shows longitudinal movements.

It is too early yet to be dogmatic about the connection between these phenomena and the normal processes that maintain the integrity and specificity of the nerve fibers. *However, it is suggestive* that the process of nerve degeneration begins by a segmentation of the fiber, first into very long ovoids, then into shorter and shorter segments, by a series of changes that strongly resemble the break- [illegible] under surface tension. I have already [illegible] possibility that the "trophic influence," which in normal life maintains the integrity of the fiber, acts by somehow resisting this tendency of surface tension to produce segmentation. We have seen that the substance of the fiber shows

large terminal bulbs, even among the cells of the cortex, where normally no nerve fibers are found.

Such experiments suggest that the diameter of a regenerating nerve fiber depends on the specific properties of its parent-nerve cell. Further work has shown, however, that this influence of the cell can become fully manifest only if the new nerve fiber also makes contact with an appropriate periphery. Thus Weiss and Taylor (1944) made regenerating fibers divide into two streams, only one of which was allowed to reach the periphery; this latter stream came to contain larger fibers than did the former. The same effect was shown by an entirely different method by Sanders and Young (1945 and 1946) and Aitken, Sharman, and Young (1947). By crushing with forceps, they interrupted the fibers of the [illegible]

[illegible] end-organs (Fig. 1). The fibers that were able to reach the periphery matured rapidly and, after 100 days of regeneration, had already begun to show the bimodally distributed fiber spectrum that is characteristic of many muscle nerves. On the opposite side, however, the fibers that were made to end blindly remained small and showed a unimodal fiber size distribution. Weiss, Edds, and Cavanaugh (1945) also showed that fibers unable to connect with their end-organs remain small.

The periphery therefore exercises an important influence on the newly formed nerve fiber during regeneration and probably also during normal development, but the means by which the effect is produced remains completely obscure.

The periphery acts upon already formed, as well as upon newly regenerated, nerve fibers. There is a considerable amount of scattered evidence that fibers and cells disconnected from their periphery become small and perhaps ultimately undergo atrophy and disappear altogether. In our experiments we have seen very marked signs of this shrinkage of fibers in the central stump of a nerve that has lost its connection with the end-organ (Fig. 1). The effect is reversible, and if such disconnected nerves are later again allowed connection with muscle fibers, then the fibers will return to their normal diameters.

DETERMINATION OF CELL SIZE

[illegible] the size of the axon produced in regen-

are already so differentiated that they are unable to produce the growth in diameter or to medullate.

Conversely, when the large medullated somatic fibers of an intercostal nerve are made to grow into the narrow tubes of the degenerated mesenteric nerve, they increase in diameter to some extent and become medullated. But the diameter of the fibers so produced is much less than that produced by the same fibers regenerating into their own peripheral stump. Evans (1947) has produced another clear example of the same sort by making somatic nerve fibers grow into the adrenal gland, where they produce large fibers and curious

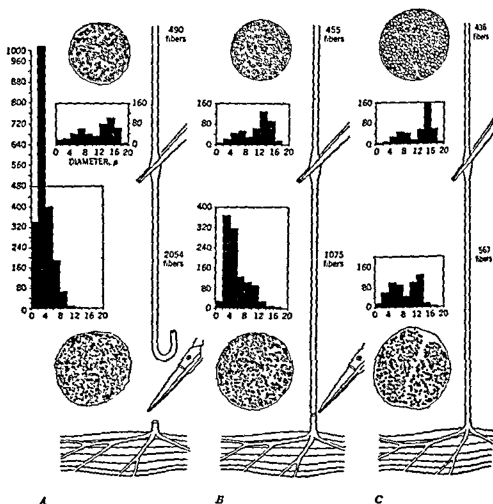


FIG. 1.—Effect of peripheral connections on regeneration. All three nerves are crushed

large terminal bulbs, even among the cells of the cortex, where normally no nerve fibers are found.

Such experiments suggest that the diameter of a regenerating nerve fiber depends on the specific properties of its parent-nerve cell. Further work has shown, however, that this influence of the cell can become fully manifest only if the new nerve fiber also makes contact with an appropriate periphery. Thus Weiss and Taylor (1944) made regenerating fibers divide into two streams, only one of which was allowed to reach the periphery; this latter stream came to contain larger fibers than did the former. The same effect was shown by an entirely different method by Sanders and Young (1945 and 1946) and Aitken, Sharman, and Young (1947). By crushing with forceps, they interrupted the fibers of the gastrocnemius nerve, on both sides of the body in rabbits; and on one side they interrupted the nerve again lower down, so that the outgrowing fibers were unable to reach their end-organs (Fig. 1). The fibers that were able to reach the periphery matured rapidly and, after 100 days of regeneration, had already begun to show the bimodally distributed fiber spectrum that is characteristic of many muscle nerves. On the opposite side, however, the fibers that were made to end blindly remained small and showed a unimodal fiber size distribution. Weiss, Edds, and Cavanaugh (1945) also showed that fibers unable to connect with their end-organs remain small.

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DETERMINATION OF CELL SIZE

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portant eleme[illegible] [illegible] size of the axon produced in regen-

eration, and hence the conduction velocity. We cannot here deal with this large question fully (see Piatt, 1948), but certain hints can be obtained from a study of the specific size differences between fibers. Since the work of Sherrington (1894) and Eccles and Sherrington (1930) it has been known that in many nerves to muscles the fibers are bimodally distributed with respect to diameter, there being peak numbers at about 8 and 15 μ of diameter (in good osmium- or Flemming-fixed preparations) and but few fibers of about 10 μ. Moreover, by cutting dorsal and ventral roots independently, these authors showed that both the large and the small fiber groups contain both afferent and efferent nerve fibers. However, some nerves to muscles show only a single peak, and Fernand and Young (1950) have recently shown that these nerves all innervate muscles outside the limbs, usually carrying little or no weight and exerting little tension. For example, the infra-hyoid muscles, facial muscles, and diaphragm are all innervated in the rabbit by these unimodal nerves, and Häggquist has shown the same for the nerve to the levator ani muscle of the cat.

A striking fact is that the modal fiber size in these cases is not that of either of the peaks found in the nerves for the muscles of the limb but is usually at about 10 μ, a diameter hardly represented in the limb nerves. Moreover, it is characteristic that nearly all the fibers lie close to this modal diameter; there are no large and few or no small fibers in the nerves. We have not established that the distribution of these fiber sizes follows strictly a Gaussian curve, but it clearly approaches much more closely to this distribution than do the fibers of the muscle nerves to the limbs. It seems likely, therefore, that in these nerves we have a single population of fibers, with a single function, coming under a common set of determining influences, whereas in the bimodal nerves four or more sets of fibers are present.

This hypothesis agrees with the fact that the muscles innervated by nerves with a unimodal fiber size distribution contain few or no muscle spindles. They are muscles that act either with gravity or at least not against it, and they have no need for the elaborate arrangement by which the muscles of the limbs adjust their tension to correspond with the weight they are called upon to bear.

The fact that the nerves in question contain no afferent fibers has been confirmed in the case of the recurrent laryngeal nerve by Evans (1950). After the vagus nerve of the rabbit central to the nodose ganglion has been severed, all the fibers of the branch of the recurrent laryngeal that enters the larynx degenerate. There is, of course, the

possibility that proprioceptor fibers leave the larynx by other pathways, but at least there are none in the recurrent laryngeal, which is a typical "unimodal" nerve, with a peak diameter at 10 μ.

It seems, therefore, that these unimodal nerves consist only of somatic motor nerve fibers. It may well be that there is a connection between the absence of proprioceptors and the fact that the largest fibers in the nerves are much smaller than in the nerves of the limb muscles. Though there is little decisive evidence about the factors that control cell size, it is likely that the amount of stimulation received by the cell is at least an important influence. Detwiler has shown that centers differentiate when afferent fibers enter them, and it may well be that the growth of every nerve cell is greatly influenced by the amount of stimulation it receives. The cells of the motor neuron pools of these muscles that have few proprioceptors presumably receive less stimulation than those that innervate muscles of the limbs; hence they reach only a lesser diameter. If this is so, it suggests that perhaps the smaller differences in diameter and hence conduction velocity are not all of functional significance.

This brief account cannot deal with all the factors that have been suggested as influential in controlling nerve-fiber diameter, but mention must be made of certain suggestions that have been put forward, notably that nerve-fiber *size* is controlled by the number of muscle fibers or weight of muscle tissue innervated. This is definitely not true for muscles of the rabbit; for example, a nerve to semimembranosus in the rabbit was found to contain 1,117 fibers, and the muscle weighed 14 3 gm., whereas the nerve to tensor fasciae cruris contained 200 fibers but the muscles weighed only 0.15 gm.; yet the fiber diameters are similar in the two nerves. The sternothyroid and an interosseous muscle of the hind limb each contains about 3,000 muscle fibers, and their nerves have about 140 nerve fibers, yet the largest nerve fibers reach 18 μ in the former but only 12 μ in the latter The eye muscles also show that the size of the motor unit (innervation ratio) is not an important determinant of nerve-fiber diameter. These small muscles have a very large number of nerve fibers, but the latter are as large as those of the limb nerves and not small, as might be expected since they control a small number of muscle fibers.

The extent of the peripheral field is therefore certainly not the only determinant of nerve-fiber size and nerve-cell size, but it is probable that it has some effect. The very fact of the peripheral influences during regeneration show this, and experiments such as

those of Terni (1920) on the lizard's tail show that nerve cells may become unduly large if they come to innervate an extended field (also Edds, 1949). Moreover, in the central nervous system there is some evidence that, other things being equal, the longer nerve fibers become the larger (Schimert, 1941); and this may be true also of peripheral fibers (Sanders and Young, 1945). In the limbs, however, the nerve fibers to the more distal segments are smaller than those to the proximal ones, because the late time of differentiation overrides the positive effect of length, and perhaps there is a conical tapering of the fibers.

In 1901 Hay showed that the nerve to the soleus muscle of the rabbit, which is composed of red muscle fibers, contains smaller nerve fibers than does the nerve for the near-by gastrocnemius, which is composed of white muscle fibers. It has since often been held that red muscle fibers, having a "tonic" rather than a "phasic" action, are controlled by relatively small nerve fibers. Examination of the nerves of other red muscles in the rabbit shows that this is a mistake. The nerve fibers to semitendinosus and quadratus femoris, for example, are much larger than those for soleus and, indeed, are as large as those for neighboring white-fibered muscles. The nerve to soleus is a special case, and its fibers are small, probably for the special reason that they come from the most caudal regions of the spinal cord. The nerves to the interosseous pedis muscles also contain smaller fibers than do other limb nerves, and they again arise from the caudal end of the cord and perhaps also taper somewhat on their long course to the periphery. It is a general rule that the fibers to proximal muscles are slightly larger than those for more distal ones in a limb.

Various minor influences are therefore at work controlling the diameter of the cells and the nerve fibers, but the major influence seems to be the degree of stimulation received by the dendrites.

DETERMINATION OF INTERNODE LENGTH

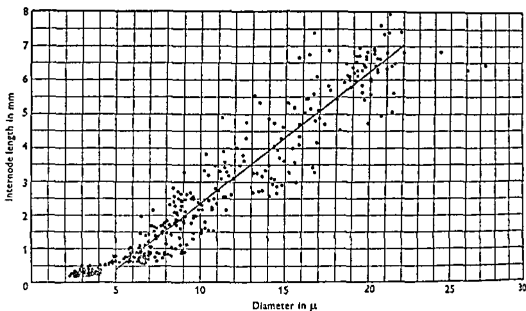
Another characteristic that varies in different nerve fibers is the distance between the nodes. Since the time of Ranvier it has been known that this distance is greater in the larger fibers. However, it is now known that internode length is not a specific characteristic of each fiber type and is not even strictly correlated with diameter. The length between the nodes is determined by the amount that the stretch of nerve in question increases in length after the time of medullation. All internodes have the same length when first laid down, about 230 μ, and the correlation with diameter that is found

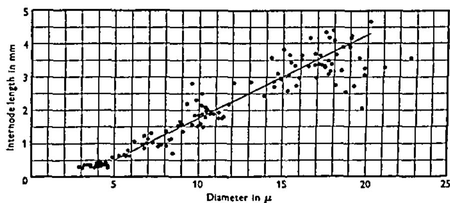
in many nerves is due to the fact that in these cases the fibers that ultimately become the largest medullate earlier and hence have the longer periods of growth.

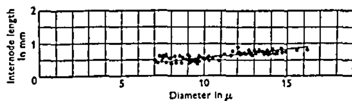
The fact that internode length is not necessarily correlated with diameter appears most clearly in regenerated nerves, where all the internodes are short, although the fibers show approximately the normal range of diameter (Young, 1946*a*, *b*; Hiscoe, 1947; Vizoso, 1950). If the above theory is correct, we should expect to find very long internodal distances in nerves lying in parts of the body that grow rapidly, and this has recently been confirmed in fishes and in man. Thomas and Young (1949) found that both in the ray and in the conger eel the internodal distances were longer in the lateral line than in the branchial nerves (Fig. 2), whereas in the electric nerve of *Torpedo*, which grows little, they were all alike. The largest fibers of these fishes have enormously long internodes—up to 8 mm.—but no internodes less than 230 μ were found. This minimum value is very striking and strongly suggests that the original periodicity is established by some relatively simple physical process, possibly surface tension. It is suggested that the early myelin, perhaps present at first in spheres, becomes arranged in very elongated, *cylindrical droplets* around the axon, pressed between the latter and the neurilemma. The length of 230 μ is certainly very long as the stable period of an ovoid that is only a few microns in diameter; it *would*, of course, be impossible for a true liquid, but the possibility cannot be excluded that very viscous liquids in a confined cylindrical space might behave in this way.

The data that Vizoso (1950) has collected show the same effect equally clearly in man. He has taken nerves from the face, arm, and leg, regions that Shepherd, Sholl, and Vizoso (1949) have shown grow at very different [illegible] length increases faster [illegible] face The minimum in[illegible] length is again 230 μ, and, as Table 1 shows, the maximum internode lengths found are about those that would be expected on the assumption that in each nerve the largest fibers become medullated at birth, with internodes of 230 μ, which were subsequently stretched in proportion to the growth of the part. The correspondence is as close as can be expected in view of the uncertainty about the stages at which medullation begins.

A curious feature in the nerves of old people is the presence of a







[illegible] n fishes. *A*, lateral-organ of *Torpedo*.

set of nodes spaced less far apart than would be expected. It is suggested that these nodes lie on fibers that have degenerated and subsequently regenerated.

TABLE 1

GROWTH OF PARTS OF THE BODY AND INTERNODE LENGTHS IN MAN FROM THE DATA OF SHEPHERD AND OTHERS, AND VIZOSO

	Lengths (In Mm)			Internode Lengths (In μ)		
	Birth	18 Years	Increase	Birth	18 Years	Increase
Lower leg	130	522	4.0×	230	990	4.3×
Forearm	139	462	3.3×	230	1,100	4.8×
Jaw	59	118	2.0×	230	550	2.4×

CONCLUSION

We are still a very long way from an understanding of the factors that give nerve cells and fibers their specific properties. The outstanding lack is of knowledge about the chemical specificities. There is, however, some information about the characteristic cell sizes and fiber diameters. Adult nerve cells retain their specificity during degeneration and regeneration, putting out large or small nerve fibers as may be appropriate. However, the large fibers acquire their full diameter only if they become connected with the appropriate end-organ. Fiber size is therefore influenced by factors acting down from the cell body and up from the periphery. This is probably true also in development but, of course, leaves us to look for another factor to explain the determination of cell size. It is suggested that a major factor in this determination is the amount of stimulation falling on the dendritic field. Thus the cell bodies and axons serving muscles that have few or no proprioceptors are shown to be smaller than those of the nerves to the antigravity muscles of the limbs, since the proprioceptors in the latter provide extra stimuli to the motor neuron pool, in addition to abundant cortical and central influences. Nerve-cell size and fiber size are not correlated with the number of muscle fibers innervated or with the character of the muscle fibers (red or white). Small systematic differences depend upon position; thus the nerve fibers arising at the hind end of the spinal cord are smaller than similar ones farther forward, and the nerve fibers to proximal muscles are larger than those to distal ones. These differences are probably not functionally significant. It is also shown that the differences in internode length between large and small fibers are not

specific properties of these fibers. All internodes are laid down with the same short periodicity; the length that they ultimately attain depends on the amount of growth that subsequently takes place. Thus the nodes are farther apart in the lateral-line nerves than in the branchial nerves of a fish and in the limb nerves than in the facial nerve of man.

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REGENERATION PHENOMENA IN HUMAN PERIPHERAL NERVES

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War has again given a stimulus to the study of injuries of peripheral nerves, and the number of significant contributions to the literature on this subject has increased greatly in recent years. Particularly has this been the case with investigations relating to degeneration and regeneration following experimentally induced traumatic nerve injury As a result of the notable experimental researches of Weiss and Young and their co-workers, valuable lessons have been learned, and much has been done to determine those principles which should guide the surgeon who attempts to assist regeneration. However, though valuable information continues to accumulate regarding the reparative process following experimental nerve injuries in lower mammals, precise information relating to many features of this process in human nerves is incomplete. Consequently, some doubt must remain as to whether or not certain experimental findings can be safely transferred to human material. Basically, nerves regenerate in the same fashion in man as they do in other mammals, but there are good reasons for suspecting that the series of events following injury of human nerves may not be precisely the same in every respect as those recorded for lower mammals. Reference to some examples will illustrate this point.

1. Severed axons are often required to regenerate over much greater distances in human nerves than in those mammals customarily used for laboratory experiments. Whether or not this modifies the process in any way requires clarification.

2. The time interval between nerve severance and repair ... may be ... of some ... could be ... but not in the former.

3. Peripheral sensory and motor mechanisms, particularly those controlling the activities of the hand and forearm, are more complex in their design and functioning in man than in lower mammals. ... complexity ... that the consec...

the result of loss or erroneous cross-shunting of regenerating axons, are more serious than is the case when less complex movements are concerned.

In this connection it should be remembered that the only reliable test of restored muscle function is the precise measurement of the response to voluntary effort. This is impossible in animal experimentation, whereas the quality of the motor recovery following the reinnervation of human muscle can be measured in this way. Moreover, in animals there are no methods for accurately testing the quality of sensory recovery, such as are available in man. For this reason human material is particularly suitable for those investigations which require precise information concerning the effectiveness of regeneration.

4. Injuries sustained in warfare and in civilian accidents differ from experimentally induced lesions, in that they are frequently more severe and are often associated with complications, such as sepsis. Under the conditions peculiar to such injuries, regeneration may not proceed in quite the same way as it does after simple crushing and severance.

5. Finally, it would seem that human peripheral nerves, as opposed to those in lower mammals, are more complicated as regards certain of their morphological features. Thus, while it is common for large nerves in lower mammals to be composed of a single funiculus, it is an unusual arrangement in man. Whether or not this morphological difference affects regeneration requires examination.

In view of the possibility that the picture of degeneration and regeneration in human nerves may depart in certain respects from that established by experimental investigations, there is an urgent need for the collection of accurate data relating to these phenomena in human nerves. The first World War provided a unique opportunity to do this, and it is unfortunate that the material which became available at that time was almost entirely neglected. The second World War has provided an opportunity to remedy this defect, and it is encouraging to note that, on this occasion, those charged with the care of these injuries have not been idle in the search for a clearer understanding of regeneration phenomena, particularly as these bear on the requirements of clinical practice. In this search they have derived considerable stimulus from the great contributions of the laboratory sciences, which should, quite appropriately, provide the biological prerequisites which are necessary for the development of improved procedures of nerve repair.

In this paper the results of certain observations on the anatomy of human peripheral nerve trunks and the course of regeneration following nerve injury will be briefly discussed. Special reference will be made to the manner in which the information provided diverges from that which has been reported from experimental investigations. The clinical material referred to in the communication has been described in considerable detail elsewhere (Sunderland, 1949).

Clinical observations covering the course and end-result of regeneration have provided data which throw light on the capacity of the axons of surviving neurons to sprout, to grow distally until they have re-established continuity with an end-organ, and to excite satisfactory function in the re-innervated tissues.

1. REGENERATIVE POWER OF NEURONS WHOSE AXONS HAVE BEEN SEVERED

Holmes and Young (1942) have shown experimentally that the surviving axons in the central stump of severed nerves retain the capacity to sprout "after nearly a year from the original injury," which was the period covered by their experiments. There is much clinical evidence scattered through the literature on peripheral nerve injuries to indicate that, in human nerves, this capacity is retained for several years. Of more importance than the capacity of the neuron to sprout, however, is its capacity to spin a new axon right to the periphery and, on re-establishing continuity with an appropriate end-organ ... This ... powe...

Information regarding the regenerative power of neurons which are sending forth new axons can be obtained from an examination of (a) the time required by them to restore a functional ... can b... ...ery which follows re-innervation. For this to be a reliable measure of their regenerative power, it is essential to have precise information relating to the quality of voluntary motor recovery, and in this regard human material possesses certain advantages which are not shared by experimental material.

A. EVALUATION OF REGENERATIVE POWER BASED ON OBSERVATIONS ON COURSE OF REGENERATION FOLLOWING PROLONGED DENERVATION

Holmes and Young (1942) have shown experimentally that "once within a peripheral stump which has been degenerated for a long

the result of loss or erroneous cross-shunting of regenerating axons, are more serious than is the case when less complex movements are concerned.

In this connection it should be remembered that the only reliable test of restored muscle function is the precise measurement of the response to voluntary effort. This is impossible in animal experimentation, whereas the quality of the motor recovery following the reinnervation of human muscle can be measured in this way. Moreover, in animals there are no methods for accurately testing the quality of sensory recovery, such as are available in man. For this reason human material is particularly suitable for those investigations which require precise information concerning the effectiveness of regeneration.

4. Injuries sustained in warfare and in civilian accidents differ from experimentally induced lesions, in that they are frequently more severe and are often associated with complications, such as sepsis. Under the conditions peculiar to such injuries, regeneration may not proceed in quite the same way as it does after simple crushing and severance.

5. Finally, it would seem that human peripheral nerves, as opposed to those in lower mammals, are more complicated as regards certain of their morphological features. Thus, while it is common for large nerves in lower mammals to be composed of a single funiculus, it is an unusual arrangement in man. Whether or not this morphological difference affects regeneration requires examination.

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TABLE 1*

THE COURSE OF REGENERATION AND THE QUALITY OF THE RECOVERY AFTER SUTURE OF THE RADIAL NERVE

Case	Level of Suture (in Cm.) above Lateral Humeral Epicondyle	Interval between Severance and Repair	Return of Voluntary Contraction in Weeks, Dating from Time of Repair: B.R	E C R	E D C	E C U	A.P.L	E.P.L	Quality of Recovery (Range and Power of Movements Have Been Expressed as Percentages of Those on Normal Side. The Value for the Range Precedes That for the Power Range Measured with a Protractor and Power with Spring Balances): Wrist Extension (with Fingers Extended)	Power of Grip	Extension of Fingers Simultaneously	Independent Extension of Fingers: Index	Middle	Little	Thumb: Radial Abduction	Extension	Residual Difference in Circumference of Forearm Measured 7.5 Cm. below Medial Humeral Epicondyle	Intervals in Weeks between Repair and Last Examination
262 I. G C	12 5	2 hours	22	27	37	36	40	45	35/50	70	Full/25	Full/25	Full/50	Full/35	70/4	Full/50	5 mm.	106
180 W. W	4 0	38 days	28	20	31	31	34	42	Full/strong	35	Full/fair				Full/weak	Full/weak	N.M.	52
166 J. C.,	I S †	207 days			9	9	18	19–25	Full/full	70	Full/75	Full/1	Full/12	Full/7	Full/5	Full/20	0 mm.	240
40 K. H McN	5 0	315 days	Intact	20	34	35	40	40	Full/full	70	Full/75	75/4	Full/35	75/5	80/35	Full/50	7 mm.	330

* B R —brachioradialis, E C R.—extensor carpi radialis longus and brevis, E D C.—extensor digitorum communis, E C.U.—extensor carpi ulnaris, A.P L.—abductor pollicis longus, E.P L.—extensor pollicis longus N M —not measured.

† I S —In supinator

time, however, fibres may proceed as rapidly as into a freshly cut one" (the period of denervation covered by their experiments was almost a year). The period for which detailed clinical data covering this feature are available is 12 months. A comparison of the course of regeneration after immediate or early repair, on the one hand, and delayed repair, on the other, indicates that, once regenerating axons enter endoneurial tubes in the distal stump, regeneration follows much the same course, regardless of the time for which the distal stump has been denervated (Table 1). Whether this desirable property is retained indefinitely remains unanswered. There is some presumptive evidence in the clinical literature that the capacity to regenerate efficient functional pathways is retained for periods longer than 12 months. Unfortunately, precise measurements of the quality of the recovery have not been recorded for those patients in whom the interval between injury and repair exceeded this limit. Furthermore, there is nothing to indicate whether the functional disability remaining in those tissues which fail to recover completely is to be attributed to the impaired regenerative power of the neurons or to some other factor. Such impairment could be due to the persistence of retrograde neuronal effects induced by the injury.

Regeneration requires the survival of nerve cells whose pathways have been injured. Following the severance of a nerve, some neurons remain unaffected, others undergo profound changes which culminate in disintegration, while an intermediate group shows a retrograde reaction of varying severity which is partly or fully reversible. Retrograde degeneration represents a permanent reduction in the number of cells which are to provide the new pathways to the periphery, while it is reasonable to assume that variations in the intensity of the reaction, the time taken for the neuron to recover, and the degree of any residual defect will affect the rate, extent, and quality of the regeneration. Unfortunately, there is no way of directly observing the extent and severity of the retrograde changes in any individual patient. Furthermore, because several factors operate simultaneously to influence the extent and quality of the recovery after nerve repair, it is impossible, where recovery is incomplete, to estimate how much of the residual defect should be attributed to retrograde neuronal changes, while compensatory mechanisms leading to full recovery in incompletely re-innervated muscles can obscure the persistence of impaired neuronal activity. Compensatory mechanisms, however, could not alone account for complete recovery. Therefore, the observation that some muscles which have been

diameter of each tube is reduced by about a half." In this connection it is of interest to note the results of an experimental investigation, in the Australian opossum, of endoneurial tube shrinkage with increasing periods of denervation (Sunderland and Bradley, 1950). Shrinkage occurred rapidly in the early stages, so that after 90 days' denervation most of the tubes were less than 3 μ in diameter, with only an occasional tube of 4 μ. Subsequent to this, there was no further significant change in the caliber of the tubes; the longest period for which the distal stump was kept denervated was 444 days. This shrinkage affected the fibers in proportion to their diameters and reduced the largest fibers to tubes which rarely exceeded 3 μ in diameter; this represents a 70–80 per cent reduction in diameter of the largest fibers.

Biopsy studies of sections of the nerve ends removed at operation for the repair of human nerves, though they fail to provide the wealth and variety of material which can be obtained experimentally, show a sequence of events which does not differ significantly from that just described. Thus, 3 months after severance, the largest endoneurial tubes in the distal segment of a severed nerve which are available for regenerating axons will rarely exceed 3 μ in diameter.

The influence of endoneurial tube size on the course and end-result of regeneration has been the subject of considerable experimental investigation (Holmes and Young, 1942; Sanders and Young, 1944; Simpson and Young, 1945; Hammond and Hinsey, 1945). From this work it was suggested that "very small Schwann tubes in the peripheral stump have a restrictive influence on fiber growth" and that the denervation shrinkage, which increases rapidly with the period of denervation, soon results in a reduction of the lumen, so that the regenerating axons which re-innervate tubes smaller than those originally occupied by them remain of reduced size. This results in the permanent impairment of the conducting properties of those fibers which fail to reach their original diameters. As a result of further investigation, however, Sanders and Young (1946) have shown that "connection with the periphery is, therefore, able to produce an increase in fiber diameter which overrides any constriction of fiber growth produced by the slightly smaller Schwann tubes." Apparently, however, these workers do not include, in the category "slightly smaller Schwann tubes," the very small tubes to which they had previously attributed restrictive influences on fiber growth. Whether or not a tube will exert a restrictive influence, therefore, hinges on the definition of the terms "slightly smaller" and "very

denervated for at least a year can recover completely indicates that the recuperative powers of some neurons are fully retained for this period.

B. EVALUATION OF REGENERATIVE POWER BASED ON QUALITY OF RECOVERY OCCURRING AFTER PROLONGED DENERVATION

Though there is clinical information to the effect that re-innervated human muscles are capable of contracting voluntarily after prolonged denervation, references to this recovery in the clinical literature are not sufficiently precise to permit an accurate end-result assessment of the quality of muscle function. For this reason the available clinical data are of no real value in deciding the maximum period of denervation which is compatible with good functional recovery.

The capacity of muscles to function efficiently following re-innervation after prolonged denervation has been studied in detail and reported recently (Sunderland, 1949). In this inquiry it has been found that very good, if not complete, restoration of function can occur in human muscles following periods of denervation of up to at least 12 months (the maximum period for which detailed clinical data are available), provided that the axons can be directed in sufficient numbers to their original, or functionally similar, end-organs and that the quiescent muscle has been maintained in the best possible condition by appropriate therapy.

C CONCLUSIONS AND DISCUSSION

On the basis of these findings it may be concluded that, in man, the capacity of some neurons to regenerate efficient functional pathways is fully retained for at least 12 months Furthermore, after periods of denervation for the same period the distal stump will receive and transmit axons in a manner which may not differ significantly from that observed when repair is undertaken immediately or shortly after severance and that very good, if not complete, restoration of function can occur in the re-innervated muscles. The implications of these findings are of interest.

Following severance of a nerve, the contents of the endoneurial tubes fluctuate, in both quantity and composition, throughout the various phases of degeneration, but ultimately they are entirely the products of Schwann cell proliferation Each tube commences to shrink with the breakdown and removal of the myelin and axon, though little information is available concerning the rate or extent to which they do so. Holmes and Young (1942) have reported that "the

former have been denervated. For reasons outlined, this is an unlikely explanation. In these cases other factors are operating which adversely influence recovery at the periphery of the limb. Briefly, conditions are more favorable for recovery in the proximal than in the distal muscles because (1) the nerve fibers supplying the proximal muscles occupy a greater cross-sectional area of the nerve at the site of suture (Sunderland and Bedbrook, 1949); (2) they are more sharply localized in the nerve at proximal levels than are the fibers destined for structures farther distally (Sunderland, 1945; Sunderland and Ray, 1948); and (3) the common action often shared by the proximal muscles assists in the restoration of function. The proximal muscles often combine as prime movers in executing movements, and for this reason the re-innervation of one member of the group by fibers originally supplying another does not seriously disturb the pattern. On the other hand, the muscles controlling the digits function as independent but well-integrated and co-ordinated systems in every movement, combining to give that delicacy, refinement, and precision of action which, in the case of the hand, is the basis of manual dexterity. In these complex and finely adjusted movement patterns each muscle has a specific role to play. Consequently, any disturbance of the fiber pattern during the regeneration of axons to these muscles seriously impairs the restoration of function.

II. RATE AT WHICH NEURONS REGENERATE NEW PATHWAYS

It is generally accepted that Tinel's sign provides evidence of the presence of "young axis cylinders in the process of regeneration," although there has been no histological confirmation of this. Assuming this to be so, an advancing Tinel sign has been employed for determining the rate of advance of axons, which has been calculated over various segments of sutured nerves at different stages of recovery (Sunderland, 1947). An analysis of the data obtained in this way has provided evidence that the rate of advance of axon tips diminishes progressively over the whole period of recovery. This is not surprising, for a steadily diminishing rate is characteristic of most growth processes. Thus the suggestion based on experimental [illegible] "there is not [illegible] distal port [illegible] is not supp [illegible] clinical investigation designed to calculate the rate of regeneration after lesions of human peripheral nerves.

It has also been shown that a relationship exists between the pro-

small." In the absence of any clear distinction between the two, the inference is that tubes not exceeding 3 μ in diameter would adversely affect the restoration of the original diameters of the large fibers.

If this were true, then nerve repair undertaken later than 3 months after severance must result in permanent immaturity of many of the regenerated fibers and, consequently, in a severe residual functional disability. However, a study of the course of regeneration and a precise evaluation of the end-results after delayed repair in human patients have revealed that the distal stump retains the capacity for at least 12 months of transmitting axons to the periphery in a manner that does not differ significantly from that observed when repair is undertaken immediately or shortly after severance and that muscle function can be fully restored following re-innervation when the distal stump has been denervated for the same period.

From this it appears that the endoneurial tube atrophy does not retard the descent of regenerating axons, nor does it prevent those changes in the restored axonal pathways which convert them into functionally efficient pathways. The precise morphological nature of the changes occurring in the regenerated fibers under these conditions remains unknown. If it represents the restoration of original fiber diameters, then, regardless of animal experiments to the contrary, the endoneurial tube shrinkage, which is maximal and severe by the third month, must be a reversible process. Under these circumstances the shrunken tubes presumably respond during regeneration to an inflating action of the regenerating axons. There is, however, some experimental evidence (Weiss, 1941, Alexander, Woods, and Weiss, 1948; Duncan, 1948, Sunderland and Smith, 1950) to suggest that it may not be necessary for the recovery of muscle function that the nerve fibers should be restored to their original diameters but that it could be due to regenerated pathways, regarded in the customary sense as morphologically immature.

In view of the considerable recuperative powers of neurons and neuromuscular mechanisms, which we have seen persist for at least a year, it would be unwise to attribute the incomplete recovery following nerve suture solely to changes consequent on a delay in re-innervation. In the past there has been a tendency to do this and, by so doing, to obscure the role played by other factors which adversely influence the quality of the recovery. Thus the poor recovery observed in the distal muscles after nerve suture at high levels (see Table 1), as opposed to *the more satisfactory recovery of the proximal* muscles, has been attributed to the longer period for which the

the initial stages of recovery is faster with high lesions, since these are close to the parent neurons, whereas, when the lesion is at a lower level, the initial rate is slower because the influence of the central forces of growth is weaker.

As a result of these findings, it is now apparent that two factors must always be taken into consideration when calculating the rate in any set of experiments, namely, (*a*) the length of nerve over which the rate is calculated, because the rate is a progressively diminishing one, and (*b*) the level at which the rate is measured, the rate for any particular segment depending on its distance from the parent neurons.

III. REGENERATION PROCESSES ADDITIONAL TO AXONAL PROGRESSION

There is evidence that the restoration of function does not follow immediately on the re-establishment of axonal continuity between the central stump and the end-organ. Thus electromyographic responses typical of re-innervated muscle can be obtained from muscles prior to the reappearance of voluntary contractions, while the direct stimulation of a nerve at operation may elicit contractions of a muscle long before it is responsive to voluntary effort.

At least three factors must be presumed to be responsible for the delay between the re-establishment of axonal continuity and the onset of voluntary contraction: (1) changes in the structure of the regenerated nerve fiber, defined as "maturation," which lead to its functional efficiency as a conducting pathway; (2) analogous changes at the end-organ, leading to effective union with muscle fibers; and (3) a minimum number of mature fibers must be present before voluntary contractions appear.

In the absence of histological examination of the nerve fiber and the end-organ during the crucial period of recovery in man, it cannot

conduction, while, according to Cajal (1928), "growth in diameter continues long after the appearance of the medullary sheath."

The following evidence suggests that further changes are required in the regenerated axon for its conversion into a functionally efficient conducting pathway.

gressive diminution of [illegible]
growing axon tips from [illegible]
when the lesion is close to the parent neurons and slower when the lesion is more remote. In the former case the rate slowly diminishes and, on reaching the more distal level, approximates the commencing rate of regeneration for a lesion in that segment.

The progressively diminishing velocity of growth which occurs throughout the process of repair could be due to the progressive decline, as the distance of the axon tip from the cell body increases, of a dynamic influence operating from within the cell body (Weiss and Hiscoe, 1948) or to a peripheral resistance which increases progressively along the peripheral stump.

It is conceivable that the shrinkage of the endoneurial tubes could contribute to the progressive diminution of the rate if the distal portion of the tube, which is denervated for longer periods than the more proximal sections, were narrower and thereby offered more resistance to the downgrowth of the axon entering it. The possibility that such a factor may be operating requires examination.

The histological picture presented by the nerve below the site of severance indicates that, after the third month, the atrophic changes, which represent maximal shrinkage of the endoneurial tubes, are substantially uniform along the entire length of the nerve. Under these conditions the argument that the tubes would be narrower distally by the time the regenerating axons reached the latter would not be valid. Despite this, a falling rate is still recorded. Furthermore, when repair of the severed nerve has been delayed for periods exceeding 3 months, the regenerating axons will enter tubes which have already suffered maximal shrinkage. If peripheral resistance is a significant factor, then it would be expected that the tubes would present a maximal resistance to the advancing axons and would therefore slow the rate below that recorded after immediate suture. On the contrary, when the course of regeneration is examined after short and long periods of denervation, there may be little difference in the time taken to cover corresponding segments of the nerve; when there is, the longer time is as often associated with the early as with the late suture.

The available evidence favors the interpretation that the diminishing rate is principally the result of waning central forces of growth, which are reduced as the axon lengthens, and that the decline is not significantly contributed to by any peripheral factor in the distal stump below the site of the lesion. Though the level of the suture does

not appear to affect the rate over any given section of the nerve, it is closely related to the initial velocity of regeneration. Thus the rate in the initial stages of recovery is faster with high lesions, since these are close to the parent neurons, whereas, when the lesion is at a lower level, the initial rate is slower because the influence of the central forces of growth is weaker.

As a result of these findings, it is now apparent that two factors must always be taken into consideration when calculating the rate in any set of experiments, namely, (a) the length of nerve over which the rate is calculated, because the rate is a progressively diminishing one, and (b) the level at which the rate is measured, the rate for any particular segment depending on its distance from the parent neurons.

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In the absence of histological examination of the nerve fiber and the end-organ during the crucial period of recovery in man, it cannot be determined in what proportion these factors are [illegible]

[illegible] growth in [illegible] long after the appearance of the medullary sheath."

[illegible]

Figure 1 illustrates the branch pattern to two adjacent muscles innervated by a peripheral nerve. In this figure A is a point on the nerve proximal to the site of origin of its branches; $y + x$ equals L^1, the shortest distance to M^1, the more proximally supplied of the two muscles, from the point A; and $y + z$ equals L^2, the shortest distance to M^2, the more distally supplied of the two muscles, from the point A.

Values for the lengths L^1 and L^2 have been obtained for the muscles innervated by the radial, median, ulnar, and sciatic nerves (Sunderland *et al.*, 1946). x^1 and z^1 are the intra-muscular distances in M^1 and M^2, respectively, which must be covered by regenerating axons before voluntary contraction is possible.

T^1 and T^2 represent the times elapsing, in days, between the suture and the appearance of contraction in M^1 and M^2, respectively. Values for T^1 and T^2 have been obtained from clinical studies of selected cases of peripheral nerve injuries.

d and D represent the initial delays elapsing before regeneration commences in axons destined for M^1 and M^2, respectively.

t^1 and t^2 are the times elapsing subsequent to the entry of axons into M^1 and M^2, respectively, and before functional neuromuscular relations are re-established and voluntary contraction is possible.

The regenerating axons, therefore, travel $(y + x + x^1)$ mm. in $T^1 - (d + t^1)$ days and $(y + z + z^1)$ mm. in $T^2 - (D + t^2)$ days.

Assuming (*a*) that the delay at A, the site of suture, is the same for regenerating axons destined for M^1 and M^2; (*b*) that the distance which must be covered intramuscularly (x^1 and z^1) and the time (t^1 and t^2) elapsing in each instance before functional neuromuscular relations are re-established and voluntary contraction is possible are identical; and (*c*) that there is no significant diminution in rate over the additional distance to the more distal muscle, then the time taken to travel $(y + z) - (y + x)$ mm. (i.e., $L^2 - L^1$ mm.) is $T^2 - T^1$ days.

For assumption *c* to be valid, it is essential to select adjacent muscles which provide low values for $L^2 - L^1$. The factors referred to in assumption *b* above are constantly present in all muscles, and, though they are not necessarily equivalent, it is reasonable to assume that if any dissimilarity does exist, it is so small in muscles of equivalent dimensions as to be unimportant. Anatomical investigation has shown that the intra muscular length of the fibers was proportional to the size of the muscles and was approximately the same in muscles of comparable dimensions. There remains, however, the possibility, which will be discussed below, that, since M^2 is denervated for a longer period than M^1, t^2 may be greater than t^1.

Application of the formula to the data provided by clinical observation reveals that the interval between the onset of contraction in any adjacent pair of muscles, $T^2 - T^1$, is greater than the time which [illegible]

the same distance. It seems reasonable to assume that the time in excess of that accounted for by axonal growth is that required for the development and completion of changes upon which efficient functioning depends. Whether the additional time is expended in converting the regenerated axon into an efficient conducting pathway, whether it represents that taken for the completion of those neuro-

Fig 1

muscular relationships upon which function depends, or whether both factors contribute simultaneously remains obscure.

If the additional time is expended in "priming" the end-organ for activity, it follows that this process must take place more slowly in the distally supplied muscle, M^2, than in the proximally supplied member of the pair, M^1; and this is consistent with the belief held by some that longer periods of denervation are responsible for accentuating those atrophic changes at the end-organ which delay the onset of recovery following the restoration of the axonal pathway.

Such an explanation appears to be unlikely for the following reason. If the duration of denervation is responsible for introducing a significant end-organ factor, then the interval elapsing between repair and the onset of recovery for individual muscles would be expected to increase with increasing periods of denervation, such as are introduced with delayed repairs. This, however, is not necessarily the case, as can be seen from an examination of data covering the onset of recovery after early and delayed repair (see Table 1). When analyzing these data and comparing the findings after early and delayed repair, it must be remembered that there is a variation, from individual to individual, in the distance to muscles (measured from a fixed point on the nerve and therefore from the site of injury) and in the initial delay at the site of repair before regenerating axons enter the distal stump. These variations influence the time of onset of recovery in individual muscles, as, of course, does the level of the injury, which also varies with the patient. These variations, however, are not such that they could be responsible for obscuring an end-organ factor by converting the course of regeneration after delayed repair into one closely resembling that following early repair.

Though the findings do not completely preclude the influence of an end-organ factor, they point to the conclusion that the additional time required to convert the fiber into a functioning pathway is consumed chiefly in effecting further changes in the regenerated axon. Whether these changes occur simultaneously along the whole length of the nerve or whether they progress along it in a proximodistal direction with a measurable velocity remains unsettled. Some information on this point, however, is provided from an examination of values for $T^2 - T^1$ for pairs of muscles which are so spaced as to provide readings over corresponding lengths of the nerve at different levels.

The interval $T^2 - T^1$ for an adjacent pair of muscles innervated at distal levels is much greater than that observed for a corresponding

length of the nerve at proximal levels. The distal muscles have certainly been denervated for a much greater period than have the proximal pair, but the following evidence indicates that this does not contribute in any significant degree to the lengthening of the interval $T^2 - T^1$ for the distal pair. After an immediate or early suture the distal pair of muscles will have been denervated for a much shorter period than the proximal pair in a patient in whom suture has been delayed for 12 months. Despite this, the value $T^2 - T^1$ for the distal pair still exceeds that for the proximal and approximates the value for the corresponding distal pair in other individuals, regardless of when the suture has been performed. From all this it may be concluded that maturation of the restored axonal pathway proceeds more slowly distally than it does proximally. Furthermore, there is evidence that myelination and the restoration of fiber diameter, which are known to influence conduction, advance down the distal stump at a later date than does growth of the axon tip.

From an analysis of all the available evidence it is tempting to conclude that the regeneration of the axon and the maturation of the restored axonal pathway, on which recovery of function depends, occur as two separate events during repair. The two processes, however, occur concurrently at first but later become distinct as elongation ceases and maturation continues. The latter involves further changes of a complex character, such as myelination and the restoration of fiber diameter.

Both restoration of the axonal pathway and maturation proceed in a proximodistal direction at a progressively diminishing rate; the latter process proceeds more slowly than does the advance of the axon. It would, however, be unwise to conclude that these processes advance without interruption or that the decline takes place smoothly along the entire length of the nerve. The movement of the axon and the process of maturation are both subject to irregular
[illegible] rate of advance measured for any
[illegible] of the
[illegible] For
this reason, when attempting [illegible] its occurring throughout the process of repair, it is advisable to examine the functional manifestations of axonal progression and maturation
[illegible] possible, since this has the ad-
[illegible] ...d
by extending the [illegible]
[illegible] g-
ments where they were not operating.

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Though the findings do not completely preclude the influence of an end-organ factor, they point to the conclusion that the additional time required to convert the fiber into a functioning pathway is consumed chiefly in effecting further changes in the regenerated axon. Whether these changes occur simultaneously along the whole length of the nerve or whether they progress along it in a proximodistal direction with a measurable velocity remains unsettled. Some information on this point, however, is provided from an examination of values for $T^2 - T^1$ for pairs of muscles which are so spaced as to provide readings over corresponding lengths of the nerve at different levels.

The interval $T^2 - T^1$ for an adjacent pair of muscles innervated at distal levels is much greater than that observed for a corresponding

V. INFLUENCE OF FUNICULAR PATTERN ON REGENERATION PHENOMENA

gage in plexus formations along the full length of the nerve. As a result of these changes, both the number and the size of the funiculi vary from level to level, while the funicular pattern alters so rapidly that transverse sections taken more than a few millimeters apart fail to present precisely the same pattern, the degree of dissimilarity depending on the length of the intervening gap (Fig. 2). The plexus for-

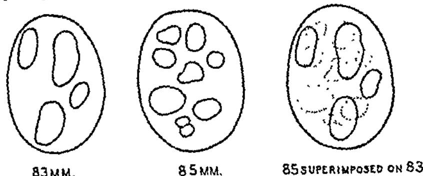


FIG 2—Transverse sections of a specimen of the radial nerve taken 83 and 85 mm above the lateral epicondyle of the humerus, to illustrate the dissimilarity in the funicular patterns produced by the funicular plexus formations occurring over the 2 mm between the sections When nerve suture is performed under these conditions, several of the funiculi of the proximal stump will be directed to the interfunicular spaces of the distal stump. Despite the dissimilarity of the funicular patterns, the chances of obtaining funicular apposition are increased when the bundles are tightly packed but are diminished when they are widely separated

mations also effect a regrouping and funicular redistribution of the component nerve fibers (Sunderland, 1945, Sunderland and Ray, 1948).

In addition to the variation in the number, size, and arrangement of the funiculi brought about in this way, a metrical analysis of the proportion of the cross-sectional area of a nerve trunk occupied by its component funiculi has shown that peripheral nerves present regional differences and peculiarities as regards this morphological feature and that the epineurial connective tissue separating the funiculi likewise varies in amount along the length of the nerve. This information has been detailed elsewhere (Sunderland and Bradley, 1949), and for the purposes of the present discussion it will suffice to state that the funiculi of human nerves are compactly arranged in some

IV. INFLUENCE OF APPROPRIATE TERMINAL CONNECTIONS ON MATURATION PROCESSES IN REGENERATED AXON

It has been demonstrated that the caliber of a regenerated nerve fiber is restored only when the growing axon re-establishes continuity with its original, or a functionally corresponding, end-organ. This suggests that the establishment of suitable terminal connections influences, though in some way as yet undefined, the maturation of the fiber (Weiss, Edds, and Cavanaugh, 1945; Simpson and Young, 1945; Aitken, Sharman, and Young, 1947).

Whether or not atrophic muscles are capable of exerting such an influence is of considerable relevance, since it is conceivable that the changes in the muscle associated with denervation atrophy may impose conditions at the end-organs which will retard or prevent the full maturation of the axons which establish connection with them. If they do, it is important to know the point at which they significantly disturb maturation of the fiber and the restoration of function. According to Aitken, Sharman, and Young (1947), the maturation of the nerve fibers in rabbit material "is as rapid when they become united with muscles allowed to atrophy for 100 or 140 days as when they grow directly into freshly denervated muscles. . . . Presumably with periods of atrophy longer than 150 days some decline in this power will become evident as the muscle fibers degenerate and disappear (Gutmann and Young, 1944)."

Two points have emerged from an investigation of the course of regeneration and the capacity of human muscles to function efficiently following re-innervation after long periods of denervation: (1) the onset of recovery in the various muscles innervated by a nerve is not necessarily retarded by delays of a year in the re-establishment of terminal connections; and (2) complete or very good restoration of function can occur following periods of denervation of at least 12 months (the period covered by the inquiry), provided that the axons are directed in sufficient numbers to their original, or functionally similar, endoneurial tubes and end-organs.

From this it is concluded that the duration of the denervation, the changes which this induces in the muscle, and the effect which these have on the maturation of the fiber do not assume significance within the first 12 months of denervation. Thus within this period either the maturation of the fibers is not adversely affected, or the failure of the fibers to recover their original diameters is of little, if any, significance.

by the connective-tissue packing, while the funiculi, being more loosely arranged, are more easily displaced within the nerve, thus reducing the effects of deforming forces. For example, clinical experience has shown that the lateral popliteal division of the sciatic nerve usually suffers grosser damage than does the medial popliteal division. The former division is usually composed of larger bundles and a smaller amount of connective tissue than is the medial, and this morphological feature may contribute to its greater susceptibility to injury.

The nerve, which is composed of numerous small funiculi, has an additional advantage when it is subjected to mechanical trauma which falls unevenly across the nerve, since under these circumstances the tendency is for some funiculi to escape while others are involved in varying degree. In partial injuries of this nature the intra-neural damage will be confined to the bundles lying within the field of the violence. The tendency, then, is for such lesions to be self-limiting. On the other hand, when the bundles are few and large, the violence is concentrated on all or the majority of the fibers comprising the nerve, while the rupture of intra-funicular vessels (which may be large in large bundles) and a fibroblastic reaction may lead to complications which can seriously embarrass large collections of fibers.

C ATROPHY OF DISTAL STUMP FOLLOWING SEVERANCE OF A NERVE

It is well known that the denervated distal stump of a severed nerve atrophies. Information concerning this atrophy is, however, confined to the general observation based on experimental findings that "shrinkage of the whole nerve varies, reaching to a maximum of one-half" (Holmes and Young, 1942) and to passing references in the clinical literature on nerve repair to a dissimilarity in the size of the nerve ends, which varies considerably from case to case even for the same period of denervation. Variations in the relative amounts of the funicular and connective tissues composing a nerve afford an explanation for the great difference in the dimensions of atrophied distal stumps after corresponding periods of denervation.

Denervation induces little change in the epineurium but a most profound reaction within the funiculus in the form of the disintegration and removal of the axons and myelin, which results in considerable atrophy of the bundle. Since the funiculi and epineurium react quite differently to denervation, it follows that the extent of the shrinkage of the nerve trunk will be greatly influenced by the percentage cross-sectional area of the nerve devoted to the funiculi

regions and are widely separated in others. Three illustrations will demonstrate the importance of this morphological variation.

A. INFLUENCE ON REGENERATION AFTER END-TO-END SUTURE

Following suture of a severed nerve, the degree of separation of the bundles becomes a factor affecting the entry of regenerating axons into the endoneurial tubes of the funiculi of the distal stump and therefore a factor influencing the extent and quality of recovery. Thus the destruction of even a few millimeters of a nerve means that the nerve ends will present dissimilar funicular patterns (Fig. 2). Despite this dissimilarity, end-to-end apposition of funiculi may still be attainable during repair and the chances of restoring useful connections consequently improved when the funiculi are tightly packed, since there will then be less interfunicular tissue into which regenerating axons may be directed. The conditions, however, are reversed when suture is performed in a zone where the funiculi are widely separated by an increased amount of interfunicular connective and/or adipose tissue. Under these circumstances the end-to-end apposition of funiculi is rendered more difficult or even impossible, and the possibility of some funiculi being unavoidably directed toward the interfunicular spaces is considerable. When this occurs, many regenerating axons enter the interfunicular spaces and there end blindly. Thus the presence of a larger amount of connective tissue results in a larger number of fibers going astray. Recovery under such circumstances is slower and less perfect and the prognosis consequently worse.

So far as can be ascertained, such variations have not been taken into account when evaluating the course of regeneration and the end-result of nerve suture in experimental animals. In such forms the nerve is often composed of a single bundle or one major bundle with several small satellites, which, as has been noted, is an arrangement which favors regeneration. This may account, in some measure, for the more favorable results reported following nerve suture in animals.

B. RELATIVE SUSCEPTIBILITY OF NERVE TRUNKS TO MECHANICAL TRAUMA

It is conceivable that nerves or those segments of nerves which are composed of large and tightly packed funiculi with little connective-tissue packing are more vulnerable to mechanical injury than those in which the bundles are smaller and are more widely separated by a greater amount of connective tissue. In the former the forces acting on the nerve are concentrated on the main content of the nerve, namely, the funiculi. In the latter they are broken up and dispersed

recovery of muscle function that the nerve fibers should be restored to their original diameters. There is some evidence to support the latter interpretation, which calls for a revision of present concepts concerning the relationship between fiber diameter and fiber function.

c) In view of the considerable recuperative powers of neurons and neuromuscular mechanisms, which persist for at least a year, it would be unwise to attribute incomplete recovery following nerve suture solely to changes consequent on a delay in re-innervation. Incomplete recovery after delayed repair demands a search for other factors which adversely influence the quality of the recovery.

2. Elongation of the axon and maturation of the restored axonal pathway, on which recovery of function depends, occur as two separate events during regeneration. The axon advances more rapidly than does maturation, but both proceed distally at a progressively diminishing rate. The two processes occur concurrently at first but later become distinct, as elongation ceases and maturation continues.

The diminishing rate is principally the result of waning central forces of growth which are reduced as the axon lengthens; the decline is not significantly contributed to by any peripheral factor in the distal stump.

Two factors must always be taken into account when calculating the rate in any set of experiments: (*a*) the length of nerve over which the rate is calculated because of its progressively diminishing character and (*b*) the level at which the rate is measured because the velocity is inversely related to the distance of the growing axon tips from the cell bodies. The initial rate is faster when the axon tip is close to the parent neuron and slower when it is more remote. In the former case the rate slowly falls and, on reaching the more distal level, approximates the commencing rate of regeneration for a lesion in that segment.

3. The influence which terminal connections exert on the further development of the fiber subsequent to the restoration of the axonal pathway is not adversely affected by delays of a year in re-establishing neuromuscular connections.

4. Human nerves are usually multifuniculated structures, in which the relative amounts of [illegible]

[illegible]

[illegible] importance of this morphological variation has been discussed in relation to its influence on the regen-

Thus the atrophy would be greater where the nerve was composed of tightly packed funiculi with little supporting connective tissue than where the funiculi were widely separated by a large amount of epineurial tissue. Consequently, the reduction in the cross-sectional dimensions of the nerve will not parallel the funicular atrophy but will show irregular fluctuations, depending on the funicular/connective-tissue ratios and the duration of the denervation.

A practical point of interest in this connection is that the funiculi and connective tissue may be so proportioned at the site of severance that the denervation changes occurring distally would, though profoundly affecting the funiculi, have little effect on the cross-sectional area of the nerve trunk. End-to-end suture under these conditions might, in the absence of a cross-sectional analysis of biopsy material, give the erroneous impression that good apposition had been obtained when, in effect, the pre-existing disparity in the funicular patterns would have been grossly accentuated by the atrophy of the bundles in the distal stump. As a result, many regenerating axons would fail to reach funiculi and their contained endoneurial tubes.

VI. SUMMARY

A study of the progress of recovery, from onset to termination, and the quality of the end-result after the repair of human nerves provides some interesting information regarding the mechanism of regeneration. From such studies the following facts have been ascertained:

1. The regenerative power of neurons which survive amputation of their axons is fully retained for at least 12 months (the period covered by detailed clinical observation). Within this period they retain the capacity to spin an axon to the periphery without any impairment of the process and to excite satisfactory function in the re-innervated tissues. After denervation for the same period, the distal stump will transmit axons in a manner which does not differ from that observed when repair is undertaken immediately or shortly after severance, while very good, if not complete, restoration of function can occur in the re-innervated muscles. From this the following conclusions can be drawn:

a) The atrophic changes developing in human muscles, which are directly attributable to denervation, are fully reversible for periods of denervation of up to at least 12 months.

b) Either the endoneurial tube shrinkage occurring with denervation is a reversible process, or, if not, then it is unnecessary for the

recovery of muscle function that the nerve fibers should be restored to their original diameters. There is some evidence to support the latter interpretation, which calls for a revision of present concepts concerning the relationship between fiber diameter and fiber function.

c) In view of the considerable recuperative powers of neurons and neuromuscular mechanisms, which persist for at least a year, it would be unwise to attribute incomplete recovery following nerve suture solely to changes consequent on a delay in re-innervation. Incomplete recovery after delayed repair demands a search for other factors which adversely influence the quality of the recovery.

2. Elongation of the axon and maturation of the restored axonal pathway, on which recovery of function depends, occur as two separate events during regeneration. The axon advances more rapidly than does maturation, but both proceed distally at a progressively diminishing rate. The two processes occur concurrently at first but later become distinct, as elongation ceases and maturation continues.

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3. The influence which terminal connections exert on the further development of the fiber *subsequent to* the restoration of the axonal pathway is not adversely affected by delays of a year in re-establishing neuromuscular connections.

4. Human nerves are usually multifuniculated structures, in which the relative amounts of [illegible] importance of this morphological [illegible] has been discussed in relation to its influence on the regen-

eration following nerve suture, the relative susceptibility of nerve trunks to mechanical trauma, and the atrophy of the distal stump of a severed nerve.

5. Attention has been directed to certain features of the mechanism of regeneration concerning which the information derived from investigations of the course of regeneration in human nerves is in conflict with that reported as the result of animal experimentation.

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It was one of the achievements of the conference to bring into focus the inherent affinities between the phenomena of differentiation, growth, regeneration, and maintenance of the nerve cell and nerve tissue. The discussions helped to integrate these diverse fields and to counteract the centrifugal tendencies of overspecialization which become apparent, once each special field has collected enough data to become self-sufficient.

The conference was fruitful in another way. Instead of reviewing past achievements, it attempted a critical evaluation of the present state of affairs. The present moment seems to be opportune for such an effort. We have the impression that, after thirty-five years of intense descriptive and experimental research, the first chapter of neuroembryology is coming to a close. It became evident during the conference that promising pioneer work is being done in the application of new and refined tools to problems which had hitherto defied analysis. At the same time, a more judicious and concise formulation of our problems and a more critical evaluation of our data are indispensable for any further advance.

The following essay is in keeping with the general aims of the conference. Rather than give a comprehensive review, we have attempted to present a critical appraisal of some basic concepts and a programmatic, rather than a historical, treatment of some current issues in our field. We have focused our attention on a few selected [illegible] . The [illegible] with [illegible] most familiar.

II. THE SCOPE OF NEUROEMBRYOLOGY

About thirty-five years ago the foundation of neuroembryology was laid under the leadership of Harrison, Detwiler, Coghill, and others They formulated the basic issues, and their extensive pioneer work led to the discoveries which are the cornerstones of our present concepts From the beginning, the analytical approach was deliberately placed in the foreground, and the methods of experimental embryology, such as embryonic transplantations, which were elaborated at about the same time, were ingeniously applied to the developing nervous system. As is well known, Harrison inaugurated the method of tissue culture in connection with problems of nerve fiber origin. The in [illegible] tributions to the sc[illegible] hands of G. Levi, O[illegible], P. Weiss, and others. The derivation

SOME ASPECTS OF NEUROEMBRYOLOGY

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The functional nerve cell is more than a conducting mechanism, whatever may be involved in conduction. It is from the beginning a dynamic system, reacting to its environment after the manner of a living organism. Physiological conduction is, so-to-speak, its accessory or secondary function. If it ever loses its potentiality of growth and differentiation, we do not know when and where.—G. E. COGHILL, *Anatomy and the Problem of Behavior* (1929), p. 84.

I. INTRODUCTION

WE HAVE witnessed in the last decades the emergence of the field of "neuroembryology" or "genetic neurology" from general experimental embryology. This development recapitulates similar events in earlier periods, when neuroanatomy and neurophysiology were emancipated from their mother-sciences. In this trend is reflected the unique position of the nervous system among the organ systems. The complexity of its structure and functional properties is foreshadowed in its embryonic development. It is, in particular, the nerve fiber, that unique product of cellular specialization, which poses as many challenging questions to the student of its differentiation and regeneration as it does to the student of its fine structure and of its electrical properties. We have in mind not merely the problems connected with the spinning-out of the nerve fibers and the formation of nerve patterns but also the complex trophic relations between nerve cells and innervated structures which are mediated by the nerve fibers. The growing and differentiating nerve center finds itself in a delicate equilibrium with its immediate milieu, with other nerve centers that synapse with it, and with the remote peripheral structures which it innervates. These threefold relations persist throughout life, and any disturbance of the equilibrium threatens not merely the normal progression of differentiation but the very existence and survival of the nerve cell. From this viewpoint, the investigation of the developing nervous system assumes a particular significance, because it reveals some basic biological properties of the nervous tissue more clearly than does the study of the adult nervous system, where problems of the conduction of impulses overshadow all others.

individual cells proliferate, differentiate, migrate, and grow, they become, at the same time, constituent parts of nerve centers, and their fibrous outgrowths form fiber tracts which have developmental patterns of their own. For instance, proliferation is not merely a matter of individual cell divisions. The mitotic cells are arranged in definite regional patterns, and they follow definite time patterns. The analysis of proliferation must take both aspects into consideration. The same holds for all other components of development.

C. Stated in a general way, the basic problems in experimental neuroembryology crystallize around the *analysis of the factors* controlling each of the components of neurodifferentiation listed above. One would investigate the conditions under which proliferation, cytological differentiation, etc., proceed in a given cell or cell group, at a given stage of development. In this procedure it is useful to distinguish between the following:

1. Intrinsic factors, residing within the cells under consideration
2. Extrinsic factors; in this category, one may distinguish:
   a) Neural factors, that is, the influences of nerve centers, fiber tracts, and synapses on neurogenesis
   b) Peripheral, nonnervous factors, such as the influence of the innervated peripheral organs on neurogenesis

D. The *tools of analysis* have been confined almost exclusively to the standard methods of experimental embryology: extirpation, transplantation, and tissue culture. The transplantation method has been, so far, the most sensitive method for the detection of the earliest differences between embryonic structures, before they can be detected in the fine structure or in the chemical composition by our present [illegible] feren[illegible] estal[illegible] the nature of the differences must await the refinement of the latter *technics*. Apart from demonstrating differences, the transplantation method carries the causal analysis of the differentiation process to a considerable depth. It can show to what extent interactions between embryonic tissues are instrumental in calling forth localized and specific differentiation processes (embryonic induction). Once such correlations are established, they can be analyzed further by appropriate variations of the implanted materials.

The material used in experimental neuroembryology has been confined largely to teleosts, amphibians, and the chick embryo. Few

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motor neuroblasts which are found in the neural epithelium of the spinal cord of young embryos are good examples. Difficulties arise when we try to distinguish between neuroblasts in more advanced stages and neurons. The same question arises with respect to many other differentiating cells: After what point should they be designated as "mature" cells, without the affix "blast"? The criteria for a decision are by no means always obvious. It is possible to give a rational and concise answer to this problem in a general way: Following the concept that the essential point in progressive specialization, or differentiation, is the synthesis of new specific cell constituents (primarily proteins) in different cell strains, one may designate a cell as an embryonic, or incompletely differentiated, cell, that is, in our instance, as a "neuroblast," as long as the synthesis of new specific constituents is still in progress. Once this process is terminated, so that further metabolism results merely in the reproduction of existing constituents, then the cell may be considered to be a mature cell, or in our case a "neuron" (see the lucid discussion of the general problem of differentiation by P. Weiss [29, 30]). This condition would coincide, in most instances, with an irreversible fixity of the cellular differentiations.

Unfortunately, our information on progressive cytodifferentiation of neuroblasts is so meager that we cannot yet apply this criterion to the actual nerve cell. At the moment we are left with the few morphological and functional criteria which we are in a position to observe in the developing neuroblast. Of these characteristics, the attainment of terminal size can be ruled out as a valid criterion. In higher vertebrates and in man the terminal size is attained in advanced stages, long after the nerve cells have become functional "neurons"; and in some teleosts, which continue to grow throughout life, there exists no fixed terminal size at all. One may be inclined to designate the stage *when the* cell begins to function as the stage of maturity; but many nerve cells reach this stage long before they are structurally complete. Our dilemma is clearly illustrated by the somatic motor cell of a 5-day *chick* embryo. Superficially, it resembles the adult multipolar neuron. It is located in its final position, and it is functional. However, the cell is immature in several respects: its Nissl substance is dispersed, the terminal connections of [illegible] neurite are provisional [illegible] until the eleventh [illegible] might call such a [illegible] "neuroblast" or a "neuron," depending on the emphasis which one wishes to place either on its structural incompleteness or on its functional activity. Clearly, more insight into

data on mammals are on record. The advantage of the amphibian material is the wide range of possibilities for transplantation experiments and, in particular, the *feasibility of extending the analysis to* the earliest stages of determination of nerve structures. The advantage of the chick embryo over the amphibian embryo is the clearer and more distinct neurological structuration of the nerve tissue and a more extensive knowledge of the normal development, based on the study of silver-impregnated material.

E. Which *criteria* are at our disposal to record the effects of an experimental situation on the developing nervous system? Our criteria are confined largely to two sets of data: the observation of *microscopic structural changes, using the ordinary staining* and silver-impregnation technics, and quantitative data, obtained by cell counts, area measurements, and mitotic counts. The silver-impregnation technics are indispensable and are, in some instances, the only valid criteria for the detection of neuroblast differentiation. The ordinary staining technics are equally indispensable for studies of mitotic activity, cell growth, and degeneration. Only a combination of both guarantees an exhaustive picture of normal, as well as experimentally modified, nerve-tissue differentiation.

III. SOME TERMINOLOGICAL QUESTIONS

A number of terms are widely used in neuroembryology and generally accepted, although their precise meaning has never been scrutinized. Among them are "neuroblast," "spongioblast," "germinal cell," "ependymal cell." The exact definition of these terms was not of crucial importance as long as neurogenesis was largely a descriptive science; but, when a refined experimental analysis is in progress, the lack of clear definitions may result in grave misunderstandings. However, the conciseness of concepts and definitions is, in turn, dependent on the factual information on hand, and our present lack of terminological precision in neurogenesis reflects, in part, the wide gaps in our knowledge in this field.

The following discussion will be focused on a few terminological difficulties which we have encountered.

A. As regards the *distinction between "neuron" and "neuroblast,"* the term "neuroblast" designates an immature or incompletely differentiated nerve cell. This term is unequivocal, as long as one deals with cells which show definite characteristics of nerve cells, such as an affinity to silver, but which are structurally incomplete, nonfunctional, and not yet located at their terminal positions. The bipolar

lines of differentiation, depending on the conditions to which it is exposed.

It will become obvious in the following discussion that not all undifferentiated cells are indifferent. It is particularly misleading to designate any cell which has no affinity for silver as an "indifferent" cell. We begin now with the discussion of the components of neurogenesis.

IV. PROLIFERATION

Our information on the proliferative process in the nervous system is very limited, although the situation is favorable for its investigation. As in other epithelia, all mitotic figures are formed at its inner surface, which is, in our instance, the inner lining of the central canal. In the ganglia, mitotic figures are dispersed. Sauer (26) has given a detailed account of the cytological details of proliferation and of the transformation of neural epithelial cells into mitotic or "germinal" cells. He has given new, conclusive evidence for the contention that the so-called "germinal cells" are mitotic stages of the neural epithelium cells and not a special type of cells, as was assumed by His. Furthermore, he has shown that the daughter-cells of a mitotic cell return to the interior of the neural epithelium immediately after they have undergone mitosis.

A. DISTRIBUTION PATTERNS OF CELL DIVISIONS

Th[illegible]
ce[illegible]
da[illegible] spinal cord of the chick embryo (16). Both investigations show clearly that mitotic activity is a patterned process. There exist patterns of distribution of mitotic figures, both in space and in time. One of the most striking features in both animal groups is the relative independence of proliferation patterns in the basal and alar plate, respectively. In the chick embryo the peak of mitotic activity in the alar plate lags behind that in the basal plate by several days. Moreover, the mitotic activity in the alar plate is consistently higher than in the basal plate, and the patterns of the dorsal and ventral halves show contrasting features during the main period of proliferation (Fig. 1). For instance, while the curve for the alar plate rises to its peak (third to sixth days), that of the basal plate declines from its peak (on the third day) to a very low level. Similar contrasts between basal and alar plates were found in the distribution patterns of the mitoses at different levels of the same embryo.

It is reasonable to expect a rather close correlation between mi-

the chemodifferentiation of developing nerve cells and into their electrical and other properties connected with function are required before we can resolve this question.

B. *The distinction between an undifferentiated cell and an early neuroblast* is equally problematical, even if we disregard all aspects of determination and limit ourselves to the cells in the neural epithelium. These cells are undoubtedly in their initial phases of differentiation and are no longer exchangeable for epidermal or somite or any other cell. Yet many of them may still be pluripotential, that is, capable of differentiation into different types of neurons or glia cells. In most instances we have no criteria which would be indicative of the transition from this stage to that of a neuroblast or spongioblast, since we cannot transplant individual cells. The situation is clear only in the few instances of very early and very rapidly differentiating neuroblasts, such as the somatic motor cells, the earliest internuncial neurons, and the exteroceptive sensory cells. In the case of the first, the cell differentiates a neurite and acquires affinity for silver shortly after it has undergone mitosis and when it has barely started its centrifugal migration. It stands out clearly against the background of neural epithelial cells, as is shown in the early pictures of Cajal of the spinal cord of a 2-day chick embryo. However, a large number of nerve cells do not follow this standard scheme, which is generally depicted in textbooks. For instance, many secondary and association neurons of the spinal cord follow an entirely different pattern. Following mitosis and for a considerable period thereafter, they remain indistinguishable from the neural epithelial cells with which they are mingled. They grow slowly, and they gradually acquire certain cytological characteristics of neuroblasts, such as an enlarged nuclear size; this happens before they acquire an affinity for silver and before the outgrowth of a neurite begins. Our present technics do not permit us to distinguish between incipient neuroblasts of this type and undifferentiated cells.

C. *The distinction between "undifferentiated" and "indifferent" cells* is a conceptual distinction which is independent of our factual information. The first term refers merely to the lack of demonstrable structural differentiation. It is relative in the sense that a refinement of our tools may reveal the beginnings of differentiation in cells which we now call "undifferentiated" (see above). The term "indifferent" refers to the potentialities of a cell which can be revealed only by experimental procedures. A cell is called "relatively indifferent" if it has the capacity to differentiate along either one of two or more

expect peaks of mitotic activity in these levels, preceding the motor column formation. Instead, we found an even distribution of ventral mitoses throughout the critical stages, and *no* sign of peaks in the limb levels (incidentally, Coghill did describe such peaks for the leg level of *Amblystoma*). What was originally described as an "unexplained discrepancy" has recently found an unexpected explanation (25). It was observed that the motor column in early stages (at 3½ days of incubation) is actually of uniform size throughout the length of the spinal cord. This finding is in agreement with the uniform distribution of ventral mitoses. The regional differences come about at later stages in the following way (Fig. 2): In the cervical level substantial numbers of motor neuroblasts degenerate after they have started differentiation and sent out fibers. In the thoracic and sacral levels considerable numbers of motor neuroblasts migrate in groups in a mediodorsal direction and establish themselves in a central position, near the central canal, as preganglionic sympathetic centers [illegible] words, the larger size of the motor columns at the limb levels is not due to a higher rate of proliferation but to the fact that they are spared the depletion to which all other regions are subjected.

The corresponding size differences in spinal ganglia originate in a *different fashion*. Mitotic counts have shown that the mitotic activity is greater in the brachial and lumbosacral than in the cervical and thoracic ganglia, from the earliest stages which we have investigated (5 days of incubation). The size differences are further accentuated by a differential degeneration of differentiated neuroblasts, which occurs only in the cervical and thoracic but not in the limb-innervating ganglia (18).

All these data show clearly that the regional differences in the sizes of nerve centers which we find in advanced and adult stages are the end-result of a combination of *different factors*. Each instance has to be analyzed on its own merits, and generalizations from one to the other are unwarranted. In particular, there is no justification in ascribing all numerical differences indiscriminately to differences in proliferative activity, as has been done quite frequently.

The correlation between proliferation and histogenesis, though valid in a general way, has its *definite* limitations. Especially in the brain, the situation would seem to be extremely complex in this respect. Each small sector of the neural epithelium probably gives rise to a large number of brain centers, and it would seem to be a

totic patterns and the subsequent histogenetic processes. In a general way the proliferative patterns do, indeed, foreshadow the sequence of histogenetic events. The relations are particularly evident with respect to the time patterns. The histogenetic differentiation of the basal plate is several days ahead of that of the alar plate, as is its

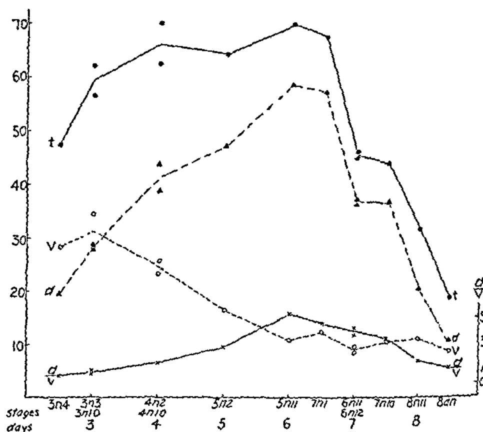


FIG. 1.—Time pattern of mitotic activity in spinal cord of chick embryo (averages for segments 10–20). *Abscissa*, stages of spinal cord differentiation and estimated chronological age; *ordinate (left)*, average number of mitoses per unit area of lining of central canal, *ordinate (right)*, ratio of mitoses in dorsal half (*d*) to mitoses in ventral half (*v*) From Hamburger, 1948, *J. Comp Neurol*, Vol 88, Fig. 5.)

proliferation. In this instance the correlations between the two events can be traced to detailed features (16).

A puzzling situation, seemingly incompatible with such a correlation, was found in the distribution pattern of basal-plate mitoses along the main axis. The neural epithelium of the basal plate gives rise, among other structures, to the motor columns. In view of the strong development of the lateral motor columns in the brachial and lumbosacral levels of the spinal cord of chick embryos, one would

futile effort to correlate the origins of specific brain centers with special details in proliferative patterns.

B MECHANISMS OF CONTROL OF MITOTIC ACTIVITY

Once certain regularities have been discovered in the temporal and spatial distribution of mitotic divisions, the question arises as to the mechanisms which control these differentials. Undoubtedly, a cell which moves toward the central canal preparatory to mitosis must be in an inner state of preparedness for division. However, intrinsic conditions alone do not seem to account fully for this event. Local conditions extrinsic to prospective mitotic cells, which would activate cells in groups, would have to be postulated to account for the shifting patterns of proliferation. Mitotic activity is a statistical event as well as the expression of an intra-cellular rhythm. We have very little information concerning these agents. They certainly do not reside in the spinal fluid because all regions of the spinal cord showing differentials of mitotic activity are equally exposed to it; besides, the patterns in the spinal ganglia could not be explained in this way. It would be reasonable to assume that mitogenetic agents would be produced in the neural epithelium or in the ganglia; however, it is difficult to imagine how such agents would remain discrete and localized within the continuous walls of the neural tube (16). Notwithstanding our ignorance in this matter, it is important to realize that any discussion of the problems of regional and topographic determination within the cord and the ganglia must include the determination of mitotic patterns which precede, and are basic to, the patterns of differentiation.

In two instances it was possible to carry the analysis a step further and to demonstrate the extrinsic control of mitotic activity. The one case is that of the spinal ganglia. Detwiler was the first to demonstrate, for *Amblystoma*, that limb extirpation results in hypoplasia, and peripheral overloading by limb transplantation in hyperplasia, of the affected ganglia, and he had ascribed these effects to changes in proliferation (10, 12). Since this interpretation was based on cell counts, it was subject to criticism. We have reinvestigated these effects in chick embryos and have established, by mitotic counts, that they are due, indeed, to modifications of the proliferative activity (18). However, it should be emphasized that peripheral factors control merely the quantitative aspects of a process which is well under way when the extrinsic control mechanism begins to operate.

The other case is that of the optic lobes in anurans. It has been

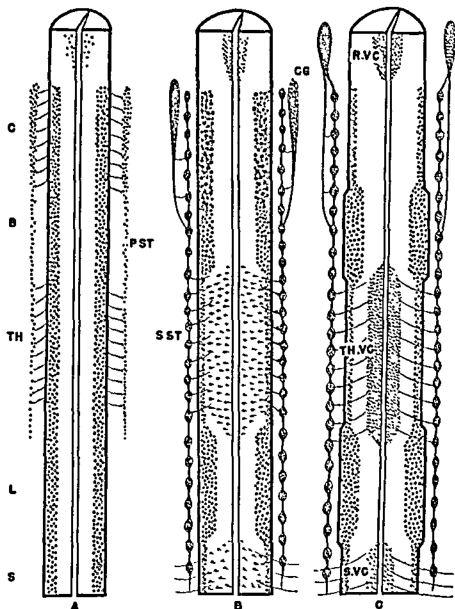


Fig. 2.—Diagrammatic frontal section of the spinal cord of chick embryos, showing the emergence of regional differences in the motor system from a morphologically uniform system. *A*, 4 days; *B*, 5 days; *C*, 8 days. *B*, brachial level; *C*, cervical level, *CG*, cervical ganglion; *L*, lumbar level; *PST*, primary sympathetic trunk, *R VC*, rhombencephalic visceral center; *S*, sacral level, *SST*, secondary sympathetic trunk, *S.VC*, sacral visceral center; *TH*, thoracic center; *TH. VC.*, thoracic visceral center (nucleus of Terni)

shown that, during this process of induction, qualitative regional differences are established in the medullary plate. At least three distinct regions are blocked out in the initial stages: anterior brain, posterior brain, and spinal cord. In amphibians these units are not irreversibly fixed; they show extensive regulative properties, following extirpation of parts (Detwiler, Harrison, and others), and they adjust themselves quantitatively when transplanted to other levels (Detwiler, 11, 12). Similar differences exist in the early chick embryo. In the 10–15-somite stages an elaborate pattern has been demonstrated. The units seem to be more rigidly fixed than in amphibians, since no appreciable regulation beyond wound-healing takes place when the lateral or dorsal half of the brachial spinal cord is removed. A detailed analysis of cases with varying degrees of defects has shown that, under the conditions of the experiment, each sector of the neural tube produces only those types of neurons which originate from it in normal development, and no others. For instance, a ventrolateral sector of the basal plate, when deprived of the adjacent tissue, will give rise only to lateral motor neurons (E. Wenger). In the same stages a regional pattern is established along the main axis. It is likewise less flexible than that in amphibians. Transplantation of the cervical or thoracic level of the neural tube to the position of the brachial cord, and vice versa, resulted in only minor adjustments to the new location. The qualitative differences which characterize these three levels were clearly expressed in their new environment. They must have been established in the primordia prior to the stage of transplantation (B. Wenger).

The neuroembryologist is largely concerned with the further elaboration of these elementary patterns in the early neural tube. The basic units which are blocked out in early stages have properties which are characteristic of similar units in other organ systems; they are usually referred to as "field properties." This concept implies that the cells or cell groups contained within each elementary unit are not yet rigidly fixed with respect to their final destination; regulation and mutual adjustments may occur within the units, mutual interactions may take place between adjacent units, and even agents extrinsic to the nervous system may control their further differentiation. The important role which the nonnervous peripheral tissues play in the development of motor and sensory nerve centers is now clearly recognized and analyzed in part. We have less information concerning the influence of developing nerve centers on secondary centers, with which they establish synaptic connections. These inter-

known for a long time that eye extirpation in early tadpoles results in a numerical hypoplasia of the optic centers in the midbrain (15, 20). Kollros (19) has obtained positive evidence that the mitotic activity is reduced in this instance.

The mechanism which operates in these cases is difficult to understand, since the affected cells have no connections of their own with the peripheral areas. We shall face the same problem in connection with the initial differentiation of neuroblasts, and we propose to defer the discussion of this point to that chapter.

It would be entirely unwarranted to generalize these findings and to assume that peripheral factors control, in general, the proliferation in all parts of the central nervous system. We have definite evidence to the contrary. For instance, one can remove the primordium of the superior oblique muscle of the chick embryo prior to the formation of the trochlear nucleus and the outgrowth of the trochlear nerve. This experiment in no way interferes with the establishment of the trochlear nucleus, which was found to be numerically complete at the advanced stage of 8 days of incubation. Not until later do degenerative processes of the neurons begin. It follows that the proliferative activity preceding the formation of the trochlear nucleus is independent of its peripheral field of termination (Dunnebacke, unpublished). The same holds for the ciliary ganglion, which differentiates normally in quantitative and qualitative respects when deprived of its terminal structure, though in later stages it shows regressive changes (Amprino).

The problem of the termination of mitotic activity is closely related to the problems discussed above. In vertebrates the mitotic *activity of the nervous system is limited to definite embryonic* periods. Our information on this point is very scanty. In the spinal cord and spinal ganglia of the chick embryo, the proliferation is practically terminated on the ninth day of incubation, although it lingers on for several more days. Is this cessation due to the fact that increasing numbers of cells undergo differentiation and therefore lose the capacity for proliferation? Or is an agent necessary for proliferation gradually being depleted? Or is it due to a combination of both factors? At present, we have no answer to these questions.

V. CELLULAR AND HISTOLOGICAL DIFFERENTIATION

The differentiation of the nervous system begins with the formation of the medullary plate under the inductive influence of the underlying mesoderm. Spemann, Holtfreter, Lehmann, and others have

shown that, during this process of induction, qualitative regional differences are established in the medullary plate. At least three distinct regions are blocked out in the initial stages: anterior brain, posterior brain, and spinal cord. In amphibians these units are not irreversibly fixed; they show extensive regulative properties, following extirpation of parts (Detwiler, Harrison, and others), and they adjust themselves quantitatively when transplanted to other levels (Detwiler, 11, 12). Similar differences exist in the early chick embryo. In the 10–15-somite stages an elaborate pattern has been demonstrated. The units seem to be more rigidly fixed than in amphibians, since no appreciable regulation beyond wound-healing takes place when the lateral or dorsal half of the brachial spinal cord is removed. A detailed analysis of cases with varying degrees of defects has shown that, under the conditions of the experiment, each sector of the neural tube produces only those types of neurons which originate from it in normal development, and no others. For instance, a ventrolateral sector of the basal plate, when deprived of the adjacent tissue, will give rise only to lateral motor neurons (E. Wenger). In the same stages a regional pattern is established along the main axis. It is likewise less flexible than that in amphibians. Transplantation of the cervical or thoracic level of the neural tube to the position of the brachial cord, and vice versa, resulted in only minor adjustments to the new location. The qualitative differences which characterize these three levels were clearly expressed in their new environment. They must have been established in the primordia prior to the stage of transplantation (B. Wenger).

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actions are of equal interest to the embryologist and to the neurologist. Space does not permit covering this field to any extent, and we shall limit ourselves to some general and critical considerations. We use the term "differentiation" in the limited sense of "cytological" or "histological" differentiation. All data refer to the chick embryo, unless stated otherwise.

A. EARLY DIFFERENTIATION ON THE CELLULAR LEVEL

The analysis is complicated by the fact, emphasized above, that neurodifferentiation proceeds simultaneously on the cellular and on supercellular levels and that both are not always separable. A unique exception are the Mauthner's cells of lower vertebrates. They occur in single or multiple pairs, and they have striking structural characteristics, among them large size and a giant neurite; hence they offer an unparallelled opportunity for the study of problems of single neuron determination and differentiation. This topic will be dealt with by other participants in the conference who have been actively engaged in their analysis.

How does cellular differentiation proceed in the neural epithelium and in the ganglia? Which are the successive steps in the progressive differentiation of the cells after they have undergone mitotic division? Our information on this topic is scanty because we have to rely mainly on the silver-impregnation technic for the detection of differentiation. We have no way of tagging the cells, and we cannot make single-cell transplantations. Tissue-culture experiments might prove to be helpful; they have been barely utilized for this particular problem.

Once a cell exhibits an affinity for silver, we are certain that it has acquired some basic properties of a neuroblast and that its differentiation is irreversibly directed toward a neuron. Usually the subsequent steps can be traced readily. The main gaps in our information are, of course, the stages between mitosis and the time at which the cell shows an affinity for silver. This span is different for different types of cells. It is very short in neuroblasts, which differentiate early and rapidly in answer to a demand for the early establishment of functional response mechanisms. The visceral and somatic motor cells, the first commissural and association neuroblasts, and the exteroceptive sensory cells in the spinal ganglia belong to this category. For instance, the motor neuroblasts begin to send out their neurites and acquire their affinity for silver very early, that is, while they migrate in the neural epithelium from the inner lining to the mantle.

Their further fate can be easily traced, and in this way we have a rather complete record from beginning to end. The same can be said of the early-differentiating neuroblasts in the spinal ganglia, which are probably tactile exteroceptive neuroblasts; they gather in the ventrolateral regions of the ganglia during the fourth and fifth days of incubation. They are recognizable very early, owing to their affinity for silver and other characteristics, such as increase of nuclear size.

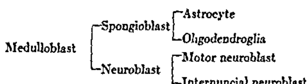
This type of rapid neuroblast formation, which is used in textbooks as the prototype for neurogenesis, is not the only one and possibly not the most common type. For instance, the cells in the spinal ganglia in the chick which do not differentiate early, that is, the ones located in the center and in the mediodorsal regions, remain small and undifferentiated for a long period. The same holds for the internuncial neurons and of the secondary sensory neurons in the alar plate of the neural tube. The precursors of these cells migrate to the mantle without showing any evidence of differentiation, and they do not acquire an affinity for silver until they have reached their terminal positions. A comparative study would probably reveal other patterns of neuroblast differentiations, all different from the standard type as represented by the motor neuroblasts. However, the differences may not be of a fundamental nature but merely shifts in the time sequence of events.

We shall comment briefly on the state of differentiation of the neural epithelial cells, preceding the first visible signs of differentiation. The neural epithelium of an early embryo contains five categories of cells: (*a*) neural epithelium cells, which are incorporated in the epithelial structure and are either prospective mitotic cells or permanent epithelial cells destined to become ependymal cells; (*b*) the same cells withdrawn from the epithelial structure and migrating toward the lining of the central canal, in anticipation of mitosis; (*c*) "germinal cells," that is, cells in mitosis (it is now generally agreed that these cells are not a special type of cells but the mitotic stages of neural epithelial cells [Sauer]; it is not known whether such a cell can undergo mitosis repeatedly); (*d*) undifferentiated migratory cells, moving toward the mantle; and (*e*) migratory cells showing visible signs of differentiation.

Categories *b* and *d* are [illegible]
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largely hypothetical and not in agreement with one another. The terms "neuroblast" and "spongioblast" refer to stages which show visible signs of differentiation. Several books have adopted the term "medulloblast" (Bailey) to designate a common precursor of the two. Unless one assumes that cells in stages *b* and *c* are already irreversibly fixed with respect to their most specific characteristics, then at least part of the undifferentiated migratory cells (*d*) would be "medulloblasts" as defined above. How long a given cell remains in this stage is unknown, and this may be different for different regions. Which pathways are open to such a pluripotent cell? Two alternatives are conceivable:

1. It proceeds through a sequence of dichotomies, such as the following:



2. It proceeds in one step from an indifferent to a highly specialized type.

There is at present no way of deciding between these alternatives. It is reasonable to assume, however, that the cells of category *d* represent a mixture of pluripotential, relatively indifferent cells and other cells which are in different phases of initial differentiation.

In view of this situation, one should distinguish sharply between "undifferentiated" and "indifferent" cells (see p. 134).

B. THE FORMATION OF CELL GROUPS (NERVE CENTERS)

Considerable variation exists in the mode of formation of nerve centers. Cells which belong to the same functional group often start their differentiation simultaneously and proceed synchronously. The cochlear, vestibular, and trigeminal centers are examples of this very common pattern; not only is it found in well-defined groups, but it occurs also in cell groups which are scattered topographically, although they may be functionally of the same type. The most striking example of the latter case is found in the reticular center of the medulla, which is spread over a wide area. Its cells start their differentiation almost simultaneously on the fourth day of incubation. In these instances supercellular controlling agents seem to play a role, and the challenging question arises as to the mechanism of synchronization.

However, synchrony is not of general occurrence. Some centers reach their numerical completion gradually, by addition of newly differentiating cells around a core of early-differentiating neuroblasts. The motor columns of the chick embryo originate in this way. It takes several days until they are completely formed. The same holds for the early-differentiating neuroblasts of the spinal ganglia. In connection with this type of growth by apposition, the idea has been developed that the early-differentiating neurons exert an "inductive effect" on adjacent, relatively indifferent cells (Barron). This topic will be taken up below.

Adjacent centers usually follow independent patterns of development; there is no evidence that one center influences the differentiation of a neighboring center. On the contrary, one observes frequently that differentiation may be in full swing in one center, whereas a contiguous cell group shows no sign of initial differentiation. The possibility of causal interrelations is, of course, not excluded by these observations

In higher vertebrates the motor columns are subdivided topographically into specialized groups. It seems that this topographic pattern originates in different ways in the few cases in which this problem was investigated. In the rat, rabbit, and chick embryos (all three are relatively rapidly developing forms) the separate columns seem to originate by the segregation of an originally homogeneous and uniform cell mass; in the more slowly developing sheep embryo, the individual cell groups originate independently and in succession (see Barron, 2).

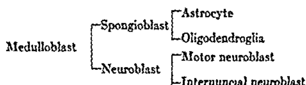
C. CAUSAL ANALYSIS

The initial differentiation of the earliest neuroblasts both in the neural tube and in the ganglia is probably due to a combination of intra-cellular conditions and activating agents located in the immediate surroundings of the cell. Unfortunately, this important step eludes experimental analysis, except in unusual cases such as Mauthner's cells, where the behavior of individual cells can be studied under experimental conditions.

The idea has been advanced that undifferentiated cell groups within the central nervous system are stimulated to neurite and dendrite outgrowth by electric currents radiating from fiber bundles which pass them on their upward or downward growth ("stimulogenous fibrillation" of Bok). This idea is refuted by a number of experiments in which segments of the central nervous system were com-

largely hypothetical and not in agreement with one another. The terms "neuroblast" and "spongioblast" refer to stages which show visible signs of differentiation. Several books have adopted the term "medulloblast" (Bailey) to designate a common precursor of the two. Unless one assumes that cells in stages *b* and *c* are already irreversibly fixed with respect to their most specific characteristics, then at least part of the undifferentiated migratory cells (*d*) would be "medulloblasts" as defined above. How long a given cell remains in this stage is unknown, and this may be different for different regions. Which pathways are open to such a pluripotent cell? Two alternatives are conceivable:

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of undifferentiated cells or on proliferation. Both instances have actually been observed (18).

It should be emphasized that almost all effects of ingrowing fibers observed so far have a rather narrow range. They call forth only moderate changes in the respective nerve centers. The bulk of the cells differentiates normally and independently of such influences. Other centers were found to be entirely uninfluenced in their early differentiation when deprived of incoming fiber tracts. This holds, for instance, for the above-mentioned cochlear and vestibular centers, with the exception of the nucleus tangentialis. The latter is unique, in that it represents the only instance showing the profound effect of fiber ingrowth on a center. The tangentialis cells in the medulla are cells which synapse with very large fibers, the "colossal fibers" from the acoustic ganglion. The tangentialis cells are first detectable as small cells in a median position; at the time when the colossal fibers enter the medulla, they migrate toward the entrance point of these fibers; they grow, and each cell applies itself lengthwise to a fiber. If the otocyst, including the primordium of the acoustic ganglion, is extirpated and the colossal fibers are thus eliminated from the start, then the tangentialis cells remain as small cells in a

e turn, finally, to the role which extrinsic, nonnervous structures play in the differentiation of the sensory and motor centers. This topic has been discussed elsewhere in detail (18) and requires only a brief comment. It is well known that the reduction of the peripheral field

extirpation,

and motor

the peripheral overloading—for instance, by implantation of a supernumerary limb—results in their hyperplasia. It was pointed out that hyper- and hypoplasia in the spinal ganglia are complex phenomena; they are the combined results of changes in proliferation, initial differentiation, cell growth, and, in the case of hypoplasia, secondary degeneration. For this reason, the observation of a hypoplastic effect can be taken as a valid proof of an extrinsic influence on cellular differentiation only if all other components are excluded. This evidence is difficult to obtain. We do not deny that hypoplasia may be due, in part, to an inhibition of cellular differentiation. All we contend is that the situation is usually not favorable for a rigid test of this point. On the other hand, the instances of hyperplasia following peripheral overloading are more conclusive. If

pletely isolated and found to differentiate normally in the absence of long-range and short-range ascending and descending fiber tracts (7, 15, 22).

On the other hand, positive evidence has been obtained to show that in certain instances the differentiation of a secondary nerve center is controlled by the ingrowth of fibers from another center with which it is in synaptic connection. For instance, the optic centers in the amphibian midbrain show a hypoplastic effect when the optic vesicles are extirpated in early stages, and they become hyperplastic if the size of the ingrowing optic nerve is enlarged. (The latter has been demonstrated by Harrison by the ingenious experiment of substituting the large optic vesicle of the large salamander, *Amblystoma tigrinum*, for the small optic vesicle of *A. punctatum*.) Similar effects can be achieved in an indirect way. For instance, one can produce first a hypo- or hyperplasia in a spinal ganglion by a reduction or an increase of the peripheral sensory area; as a consequence, the secondary sensory centers in the dorsal columns of the spinal cord show hypo- or hyperplasia, respectively.

It is necessary to exert a great deal of discretion in interpreting the hypoplastic effects observed in such experiments. Only in rare instances can the numerical hypoplasia be taken as conclusive evidence that the differentiation of undifferentiated cells was blocked and not some other developmental process. We have shown elsewhere (18) that the numerical hypoplasia of a nerve center can be the result of factors other than inhibition of differentiation; it can be due to a reduction of the proliferative activity or to a secondary degeneration of fully differentiated cells. All three types of effects were actually observed in the spinal ganglia of the chick embryo under appropriate experimental conditions. The danger of overlooking a secondary degeneration is particularly great if the hypoplastic effects are recorded a long period after the extirpations and if no intermediate observations were made. In several instances we have followed the effects of the elimination of primary centers on secondary centers over a prolonged period and in a closely timed series, and we have actually observed that the secondary centers came, at first, to full differentiation and underwent regressive changes later on (vestibular and cochlear centers, following otocyst extirpation [24]). The interpretation of cases of cellular hyperplasia is also ambiguous. If the number of fully differentiated cells is larger than normal, then we may deal with an influence of ingrowing fibers on the differentiation

of undifferentiated cells or on proliferation. Both instances have actually been observed (18).

It should be emphasized that almost all effects of ingrowing fibers observed so far have a rather narrow range. They call forth only moderate changes in the respective nerve centers. The bulk of the cells differentiates normally and independently of such influences. Other centers were found to be entirely uninfluenced in their early differentiation when deprived of incoming fiber tracts. This holds, for instance, for the above-mentioned cochlear and vestibular centers, with the exception of the nucleus tangentialis. The latter is unique, in that it represents the only instance showing the profound effect of fiber ingrowth on a center. The tangentialis cells in the medulla are cells which synapse with very large fibers, the "colossal fibers" from the acoustic ganglion. The tangentialis cells are first detectable as small cells in a median position; at the time when the colossal fibers enter the medulla, they migrate toward the entrance point of these fibers; they grow, and each cell applies itself lengthwise to a fiber. If the otocyst, including the primordium of the acoustic ganglion, is extirpated and the colossal fibers are thus eliminated from the start, then the tangentialis cells remain as small cells in a median position; they fail to differentiate, to grow, and to migrate, and they are not detectable at later stages (24).

We turn, finally, to the *role* which extrinsic, nonnervous structures play in the differentiation of the sensory and motor centers. This topic has been discussed elsewhere in detail (18) and requires only a brief [illegible] of the limb-innervating sensory and motor centers and that the peripheral *overloading*—for instance, by implantation of a supernumerary limb—results in their hyperplasia. It was pointed out that hyper- and hypoplasia in the spinal ganglia are complex phenomena; they are the combined results of changes in proliferation, initial differentiation, cell growth, and, in the case of hypoplasia, secondary degeneration. For this reason, the observation of a hypoplastic effect can be taken as a valid proof of an extrinsic influence on cellular differentiation only if all other components are excluded. This evidence is difficult to obtain. We do not deny that hypoplasia may be due, in part, to an inhibition of cellular differentiation. All we contend is that the situation is usually not favorable for a rigid test of this point. On the other hand, the instances of hyperplasia following peripheral overloading are more conclusive. If

pletely isolated and found to differentiate normally in the absence of long-range and short-range ascending and descending fiber tracts (7, 15, 22).

On the other hand, positive evidence has been obtained to show that in certain instances the differentiation of a secondary nerve center is controlled by the ingrowth of fibers from another center with which it is in synaptic connection. For instance, the optic centers in the amphibian midbrain show a hypoplastic effect when the optic vesicles are extirpated in early stages, and they become hyperplastic if the size of the ingrowing optic nerve is enlarged. (The latter has been demonstrated by Harrison by the ingenious experiment of substituting the large optic vesicle of the large salamander, *Amblystoma tigrinum*, for the small optic vesicle of *A. punctatum*.) Similar effects can be achieved in an indirect way. For instance, one can produce first a hypo- or hyperplasia in a spinal ganglion by a reduction or an increase of the peripheral sensory area; as a consequence, the secondary sensory centers in the dorsal columns of the spinal cord show hypo- or hyperplasia, respectively.

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leg buds of chick embryos, were based on the same reasoning. He found that mouse sarcoma 180 was innervated by nerve fibers which seemed to be primarily sensory in nature.

The control of the *growth* of neurons by peripheral fields has never been carefully analyzed in experimental situations. However, the extensive comparative investigations of G. Levi (summarized in Ref. 21) have clearly established such a correlation for normal development. Particularly striking examples are found in certain teleost species which apparently grow continuously. Extreme size differences between young and old specimens were accompanied by corresponding size differences in homologous neurons. Similar correlations were found between limb- and trunk-innervating neurons within the same animal and between homologous neurons of large and small species. Levi has emphasized the unique position of the nervous tissue in this respect: Since proliferation terminates long before body growth ceases, continued growth of the neurons is necessary, in order to meet the demands of the enlarging peripheral area. Thus the terminal size of neurons is controlled by extrinsic conditions.

VI. MIGRATION

Cell migration is of universal occurrence in the development of the nervous system. Although this phenomenon is an integral part of nerve-cell differentiation, we give it special treatment because it plays an important role in the pattern formation of nerve centers. We shall not discuss the migrations of the neural-crest cells and of their derivatives. All cells which remain within the neural tube migrate twice, and some of them migrate three times; they change their direction in each new migratory phase. We may distinguish three types of migratory movements: (A) *centripetal migration* of individual, undifferentiated cells toward the inner lining of the central canal, preparatory to mitosis; (B) *centrifugal migration* of individual cells toward the mantle, and (C) *group migration* of nerve centers.

A. CENTRIP[illegible]

Centripetal migrat[illegible] fibrillar stage: it is i[illegible] up with mitotic division. It was pointed out above that "germinal cells" are no longer considered to be a special type of cells but the mitotic phases of neural epithelial cells. At any given moment some epithelial cells withdraw from the epithelium, assume a spherical shape, and migrate toward the central canal. This process has been described in detail by Sauer (26).

cell counts of differentiated cells reveal a numerical increase compared with the control side, as is actually the case in ganglia and motor centers, then the conclusion seems to be justified that extrinsic factors control the transformation of undifferentiated cells into neuroblasts (for details see below).

The mechanism by which this effect is brought about is difficult to visualize. It must be an indirect effect because the undifferentiated cells which are the responding elements have no connection of their own with the periphery. The situation here is the same as in the case of the peripheral control of proliferation. In both instances the most satisfactory hypothesis is the assumption that the pioneer fibers, which grow out irrespective of peripheral changes, are mediators in the chain of events. The experimentally produced decrease or increase of the peripheral area would alter the physiological condition of the pioneer fibers which strike out into this region; they would be either prevented from further growth and branching or stimulated to excessive growth and branching. The physiological conditions of the fiber would then be reflected in the metabolism of its perikaryon. As a result, the release of metabolic products into areas adjacent to the perikarya of the pioneer fibers or the uptake of substances from adjacent areas would be decreased or increased, respectively, and this change in the constituents of the cellular milieu would, in turn, affect the differentiation process and proliferation of adjacent undifferentiated cells. Barron (2, 3) has independently developed similar ideas and implemented them with the suggestion that dendrites from early-differentiating neurons spread between the adjacent indifferent cells and stimulate their differentiation. All these ideas are admittedly hypothetical and await experimental tests. Another suggestion can be ruled out: The "peripheral control" cannot be understood in terms of increased or decreased functional demands, because hypo- and hyperplasia are observable before functional activity begins.

The "specificity" of the correlations between nervous and nonnervous structures is a problem of particular interest which occupies our attention at the moment. Some indirect evidence suggests strongly specific effects of effector organs on the development of motor centers and of receptor organs on the development of sensory centers. We try to analyze this point by exposing outgrowing mixed nerves to homogeneous tissue, such as muscle primordia, rather than to heterogeneous structures, such as limb buds. The experiments of [illegible] in which he transplanted mouse tumors in the place of

ture and withdrawal of this process occurs eventually, and the cell becomes temporarily a "unipolar" cell.

In contrast to the type of migration to be discussed below, this centrifugal migration is one of individual cells. We do not know how they manage to squeeze through the neural epithelium, and we have no information concerning the factors which direct their migration and their aggregations in the mantle.

C. GROUP MIGRATION OF NERVE CENTERS

Whereas the first two types of migration have been recognized for a long time, the third type has received little attention so far. Yet it is a striking phenomenon and of wide occurrence. A surprisingly large number of cell groups do not come to rest at the end of their centrifugal migration; they set out on a new trek, this time not as individuals but in groups. The main significance of this type of migration lies in the fact that it has a considerable share in the establishment of the definitive patterns of nerve-center arrangements.

Group migration differs in several characteristics from the previously described types of migration, apart from the fact that it concerns entire cell groups. The migrating cells are invariably full-fledged neuroblasts (or neurons, depending on the definition; see above). Their nuclei are large, and the amount of cytoplasm is reduced; their cell bodies are spindle-shaped and "streamlined" for the voyage, with the dendrites apparently merged into one, which is antipolar to the neurite. The neurites of these cells have invariably reached the periphery, and they may even have established preliminary connections with nonnervous peripheral structures. This connection is retained during the migration, which is in a direction opposite to the neurite. Since the latter is "trailing" behind, we must assume that it lengthens proximally while the cell migrates.

The best-analyzed case is that of the preganglionic visceral center in the thoracolumbar level of the chick embryo (nucleus of Terni), [illegible] from a ventrolateral to a mediodorsal position during [illegible] (95, see Fig. 3). In mammals [illegible] observations of R. Leve[illegible] [illegible] ion are the accessory nucleus of the abducens; one of [illegible] belonging to the oculomotor nucleus, which moves across the mid-line; the Purkinje cells; the cells in the optic lobe and in the retina.

The movements are always in groups, though not necessarily in compact clusters. The mechanism of locomotion of these cells, as

The same author has emphasized again what was already known to Altmann, that this type of migration is not peculiar to the neural epithelium; mitosis follows this pattern in all tubular epithelial structures. For instance, we find similar pictures in the embryonic intestine and kidney tubules. We do not know whether all neural epithelial cells participate in mitosis and, consequently, in this type of migration. According to the standard account in textbooks, some residual epithelial cells never leave the epithelium, and these cells are transformed eventually into ependymal cells. However, there seems to be little evidence for this statement. It is conceivable that some of the daughter-cells of mitotic divisions, instead of migrating into the mantle, re-enter the epithelium and again become true epithelial cells. Such a reintegration of cells occurs in all other epithelia, and there is no reason why it should not occur in the neural epithelium. If this holds true, then a distinction between potentially mitotic cells and residual, stationary cells—that is, potential ependymal cells—becomes very tenuous. It is more reasonable to assume that all neural epithelial cells have equal potentiality and equal chances for migration and proliferation rather than to postulate a dichotomy of cell types in the early tube.

B. CENTRIFUGAL MIGRATION TOWARD THE MANTLE

Centrifugal migration is again a universal phenomenon; it is characteristic of all cells which have undergone mitosis. Different cells, however, are in different stages of cellular differentiation while they migrate centrifugally. It was pointed out above that some cells show a very precocious affinity to silver; they reach the bipolar stage and develop a neurite while they are still close to the lining of the central canal. Motor neuroblasts illustrate this type. Other cells, such as the dorsal cells of the spinal cord, show no sign of differentiation while they migrate. Obviously, the mechanism of migration is not related to the state of differentiation of a prospective nerve cell.

Many of the earliest-differentiating neuroblasts in the neural tube show a so-called "bipolar" configuration when they are first detectable by silver impregnation. Their proximal process adheres to the inner limiting membrane and is probably nothing but the drawn-out proximal part of the cell body. The cell resembles the cells in the upper lip of the blastopore or the medullary plate cells, which become flask-shaped when moving away from the surface. A rup-

ing with one specific, well-circumscribed process but with a variety of different physiological mechanisms.

We wish to limit our discussion to one point, namely, the repercussions on nerve cells of changes at the periphery which are established experimentally before the tips of outgrowing fibers have reached the peripheral areas. The extirpation and transplantation experiments of limb primordia in 2½–3-day chick embryos, that is, before nerve outgrowth is well under way, establish such conditions. So far, we have studied in detail only the effects of limb-bud extirpations on brachial spinal ganglion cells. The early differentiation of considerable numbers of the large, ventrolateral neuroblasts which we assume are exteroceptive tactile neurons is not impeded by this disturbance; the beginning of fiber formation proceeds normally for about 2 days. However, shortly thereafter, that is, at a time when the fibers reach the experimentally reduced area but long before they have established their definitive connections with epidermal sense organs (which are formed much later), a dramatic degeneration process afflicts the perikarya; it reaches its peak at 5–6 days of incubation, during which stages large numbers of degenerating cells were observed and, among them, macrophages, which remove the debris (18). At 7 days a fairly static condition is reached; however, the surviving cells have an atrophic appearance at stages near hatching. It was not possible to determine whether they undergo shrinkage or whether they merely fail to undergo further growth.

This rapid breakdown affects only the early-differentiating large neuroblasts of the ganglia. Another type of ganglion cells responds in a different fashion to the limb extirpation. We refer to the mediodorsally located cells which normally remain conspicuously smaller during a long period. These cells show merely an atrophy; but no actual breakdown was observed, nor was their number reduced.

The disappearance of neuroblasts under the impact of the reduction or complete elimination of their peripheral fields was found in other instances. If the primordium of the superior oblique muscle is removed by the extirpation of the early optic vesicle and of adjacent regions, no immediate effect on the nucleus of the trochlear nerve is noticed. It is found to be numerically complete at 5 days of incubation (see above). Hence the early differentiation is not affected by the operation. In subsequent stages, however, a progressive loss of cells occurs, as was established by cell counts. More than half the cells have disappeared at 10 days, and almost 80 per cent at 15 days (Dunnebacke, unpublished). A similar situation was found in the

well as the forces which direct them to their terminal positions, is unknown. It would be of great interest to study the behavior of these cells in tissue culture and to compare it with that of stationary cell groups.

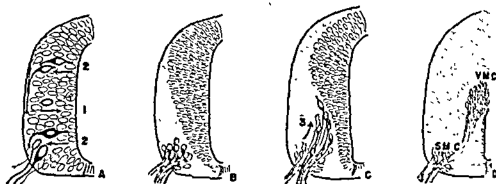


FIG. 3.—Diagram to illustrate the migration of cells in the spinal cord of the chick embryo *A*, 2 days; *B*, 4 days, *C*, 6 days; *D*, 8 days. *1*, centripetal migration to the inner lining of the central canal; *2*, centrifugal migration of internuncial and motor cells toward the mantle. In *B*, the uniform motor column is formed (see Fig 2, *A*) In *C*, the group migration of visceral preganglionic cells (*3*) begins. In *D*, the stationary cells have formed the somatic motor column (*SMC*), and the migratory cells have formed the visceral motor center of Terni (*VMC*).

VII. MAINTENANCE

A. THE ROLE OF PERIPHERAL CONDITIONS

The outgrowth of the embryonic nerve fiber into the peripheral structures and the establishment of peripheral connections open a new chapter in the life-history of the nerve cell. The cell reaches out into a new sphere of influence and of functional activity, and, at the same time, it comes under the control of the conditions to which the growing tip of its tenuous process is exposed. The cell acquires, so to speak, a second milieu, and at least some embryonic nerve cells are more sensitive to changes in their "remote" milieu than to the immediate milieu surrounding their perikaryon. To maintain the delicate equilibrium with the nonnervous structures with which it is connected is a matter of prime importance for the nerve cell throughout its life. As is generally known, an interruption of the peripheral connections results in an immediate damage to the perikaryon, and it may lead to its breakdown under adverse peripheral conditions. These interrelations are mutual. The maintenance of receptor and effector organs is, at least in some instances, dependent on their innervation; one of the most striking examples is the effect of denervation on muscles. These correlations are usually combined under the heading of "trophic" relations; however, we are certainly not deal-

environment, and one could not very well speak of a "trophic" effect. An alternative hypothesis would assume that the medium in which the neurite grows provides the latter with some substance necessary for its further growth. In this case we would be dealing with a true "trophic" relation. The two hypotheses are not mutually exclusive.

A more careful analysis of the distribution and fate of the outgrowing fibers under these adverse conditions would be desirable. Furthermore, a study of the reverse effect, namely, hyperplasia resulting from a peripheral overloading, might throw new light on this problem. So far, we have not made measurements of neurons under these conditions. However, a remarkable case of cellular hyperplasia in a similar instance was reported by Terni (28). It is known that the regenerated tail of a lizard contains no central nervous system. It is innervated by the terminal segments of the cord near the amputated surface. Terni found a threefold cellular hyperplasia in the sensory neurons which innervated the regenerated tail.

At this point we wish to mention briefly an observation which was made on normal spinal ganglia. A large-scale degeneration occurs regularly in the cervical and thoracic spinal ganglia of the chick embryo, whereas the brachial and lumbosacral ganglia are not affected (18). This phenomenon bears a striking resemblance to the experimentally induced degeneration in brachial ganglia following wing extirpation; in both instances, only the early-differentiating, large, ventrolateral ganglion cells are affected; in both instances these cells break down after they have formed their neurites; and the period during which the breakdown occurs is the same, namely, at 4-6 days of incubation, with a peak at 5 and 6 days. Future experi-[illegible]

[illegible] whether the normally occurring degeneration process is due to peripheral factors or to factors intrinsic in the spinal ganglia. A similar phenomenon of neuron degeneration was found in motor neuroblasts of the cervical region of the spinal cord of 4-4½-day chick embryos. The reasons why the degenerative process is limited to this particular level have been discussed elsewhere (25).

B THE ROLE OF SYNAPTIC CONN[illegible]

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brachial sympathetic chain ganglia, following wing extirpation (Simmler), and in the ciliary ganglion (Amprino). The latter case, which is of particular interest, will be discussed below.

In the analysis of these phenomena, it is difficult, if not impossible, to make a sharp distinction between the blocking of the progressive phase of development (differentiation, cell growth, etc.) and regressive changes in the form of atrophy. When a cell breaks down completely before reaching its mature state of differentiation, then both effects are obviously combined, and the distinction is meaningless. If a cell shows an atrophic condition, when compared with cells of the unoperated side, then, again, both effects may be combined, or we may be dealing merely with an inhibition or retardation of further growth. It would take very elaborate measurements of cell sizes, over a prolonged period, to decide between these alternatives.

In summary, we may state that the maintenance of the integrity of primary sensory and motor neuroblasts depends on appropriate conditions for the growth of its neurite. The term "maintenance" is used in a rather broad sense; it includes the maintenance of the process of differentiation and the maintenance of a dynamic metabolic equilibrium which guarantees the structural integrity of the cell.

How can one conceive of the role of the "remote milieu" in these processes? It is certain that the effects are not brought about by the failure of the neurite to establish functional connections with the peripheral structures; nor can atrophy and degeneration be considered as a "functional atrophy," that is, due to a lack of impulse transmission. The repercussions on the perikaryon are noticeable before even provisional terminations could have been established with the primordia of receptor or effector organs, had the periphery been left intact. The cellular breakdown is the result of conditions which prevented its neurite from the establishment of terminations.

Any interpretation of these interactions has to take into account that no direct injury to the neurite is involved. The fibers grow out in a normal fashion, and one frequently finds that they form a neuroma-like structure near the cut surface of an extirpated limb bud. It is conceivable that the continued spinning-out of axoplasm at the growing end-tip of a young neuroblast is a necessary prerequisite for the healthy growth of the cell and that an interference with its normal terminal branching, that is, with a high rate of outflow of axoplasm at the tip, would eventually upset the metabolic equilibrium of the entire cell and cause its breakdown. This hypothesis does not imply an exchange of materials between the neurite and its

environment, and one could not very well speak of a "trophic" effect. An alternative hypothesis would assume that the medium in which the neurite grows provides the latter with some substance necessary for its further growth. In this case we would be dealing with a true "trophic" relation. The two hypotheses are not mutually exclusive.

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B. THE ROLE OF SYNAPTIC CONNECTIONS

No nerve cell or nerve center is an isolated unit; each neuron is in synaptic connections with one or several other neurons. The question

has arisen whether the synaptic boutons, in addition to their role in transmitting nerve impulses, may be of importance for the maintenance of the neuron with which they connect. Transneuronal effects following the transection of intra-central fiber tracts have been known for a long time. They have been briefly reviewed by Bodian (6). This author has described an interesting case of such effects, namely, the degeneration of thalamic centers in the opossum following decortication (5).

Can similar effects be observed in neuroblasts if the initial ingrowth of fibers is blocked or, in other words, if a cell is prevented from ever receiving a synapse? In general, it is difficult to obtain concise data on this point because each neuroblast usually receives *several synapses from different sources and little is known of the* establishment and number of synapses in embryonic nerve centers. Furthermore, repeated cell counts over a long period or the observation of degenerating cells is necessary to establish this point. Earlier experiments which seemed to demonstrate the existence of such correlations in embryos cannot be considered as valid evidence.

The cochlear and vestibular centers in the medulla of the chick embryo have provided favorable material for such a study (24). Their synaptic connections are fairly well known; some of them receive synapses from one nerve center and others from two or more, in addition to their connections with the cochlear and vestibular root fibers. The nucleus tangentialis (vestibular) is an exception. This nucleus, which occurs only in teleosts, reptiles, and birds, synapses exclusively with a certain bundle of vestibular root fibers, the so-called "colossal fibers," and apparently receives no synapses from any intra-central fiber tract. The simple expedient of extirpating the otocyst which contains the primordia for the acoustic and vestibular ganglia deprives all secondary centers of one of their synaptic connections. The effects were found to be different for the different centers. All cochlear centers started their differentiation normally and carried on to the eleventh day of incubation. From then on, further differentiation was blocked, and regressive changes took place, including the disappearance of neurons. The degree of hypoplasia was characteristically different in different centers. The data suggest a quantitative correlation between the degree of hypoplasia and the number of synaptic connections which the cells receive. Centers which receive synapses from several sources are not severely affected if only one of them is eliminated. Centers which normally receive two synapses suffer severely if deprived of one. The (vestibular) nucleus

tangentialis, which synapses only with root fibers and apparently with no others, does not even start to differentiate if the colossal fibers fail to enter the medulla (see above). All other vestibular centers show no responses to the absence of root fibers, presumably because each cell is "protected" by numerous synaptic boutons from intra-central sources. There is a striking parallelism between these data and observations of Bodian (5) on the thalamus of the opossum following decortication, where similar quantitative correlations seem to exist between the degree of damage to nerve cells and the number of synaptic boutons which were eliminated.

In summary, there can be no doubt that in numerous instances the presence of synaptic boutons on a neuroblast is a necessary prerequisite for its normal growth, differentiation, and maintenance. We should be cautious, however, in making generalizations. For instance, the sensory cells of the spinal ganglia differentiate and maintain themselves in the absence of synaptic boutons.

Since most neurons receive synaptic connections from several sources, it is difficult to obtain nerve centers whose differentiation could be observed in the absence of all incoming fiber tracts. In the one instance which was described above, namely, that of the nucleus tangentialis, this center failed even to begin its differentiation if its one and only source of synaptic connections, the colossal fibers, was prevented from reaching the medulla. However, we are again cautioned against premature generalizations. First, the nucleus tangentialis is exceptional in other respects; furthermore, there exists a parall-

... It would be very desirable to obtain more data concerning the interesting problem of transneuronal trophic relations during neurogenesis.

The breakdown of ...
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ways lead ... in this case and in the present case of synaptic failure are the same. It was possible to study this problem in a particularly favorable case, namely, the ciliary ganglion (23). The neurons of this ganglion receive only one afferent synapse, from the visceral motor nucleus of Edinger-Westphal. This connection can be eliminated by the extirpation of the midbrain, in early stages, before the fibers of this nucleus grow out. On the other hand, the early extirpation of the optic vesicle removes the peripheral field of dis-

tribution of the ciliary nerve before it enters it. The onset of differentiation of the ciliary ganglion was not hampered by either one of the two operations. However, in both instances the majority of the cells broke down eventually. A detailed analysis of the responses following each operation and continued observations of the ganglion cells over a long period have shown that the histologic picture of the regressive changes is different in the two experiments. In the case of eye extirpation, degeneration of ciliary cells begins shortly after the onset of their differentiation. It proceeds very rapidly and involves practically all cells. The few surviving cells which appear to be normal in structure at late stages have established atypical peripheral connections. Midbrain extirpation, on the other hand, does not interfere with the differentiation of the ciliary ganglion up to 10 days of incubation, when it is almost completed. From this stage on, a progressive atrophy sets in, which results eventually in cellular breakdown. By the time of hatching, the majority of the cells have disappeared; the few surviving cells are highly atrophic, in contrast to the survivors of the first operation. Obviously, the metabolic disturbances resulting in cellular breakdown are different in the two instances. The integrity of one mechanism does not "protect" the cell against detrimental effects resulting from the disturbance of the other.

In concluding, it may be well to point out that nerve cells in tissue culture are capable of survival and growth in the absence of all synaptic contacts both at the periphery and at the perikaryon. Obviously, we are dealing here with atypical nutritional conditions. It is conceivable that the culture medium provides all the necessary conditions for fiber outgrowth and branching and that competition for nutritive resources is eliminated. The application of the tissue-culture method to the problems discussed in this chapter might be of considerable aid in the elucidation of the physiological mechanisms involved and fruitful for the study of differential susceptibilities and metabolic requirements of different types of neurons.

VIII. CONCLUSION

The trophic relations between the nerve cell and its nonnervous peripheral milieu are of fundamental importance for the integrity of the nerve cell not only during its embryonic phase but throughout its life. The repercussions of nerve transection on the perikaryon and the dependence of its complete restoration on the re-establishment of normal terminal connections will be discussed elsewhere in this

volume. Likewise, transneuronal effects are manifest in embryonic and adult stages. Finally, it should be pointed out that the relations between nervous and nonnervous structures are reciprocal, as is strikingly demonstrated in the regressive changes observed in denervated musculature.

The interactions between nerve cells and nonnervous structures are not "functional" effects, that is, effects due to the inhibition of impulse transmission, but are "trophic" or metabolic effects. The elucidation of the physiological mechanisms involved is one of the goals of neuroembryology.

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STUDIES ON THE DEVELOPMENT OF MAUTHNER'S CELL

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DURING the course of my research on the static centers of vertebrates, my attention was attracted by the Mauthner's cells of amphibians. Their giant size, constant shape, and precocious localization seem to make them the most suitable material for research on the histogenetic determination and differentiation of nerve cells.

At what stage is a highly specialized neuron, such as the Mauthner's cell, determined? Do neuroblasts acquire all future differentiation through external influences, or do intrinsic factors affect their differentiation? These are problems of pre-formation and epigenesis translated into cytological terms.

When I started my research on Mauthner's cells, very little experimental work had been done in this field, and little was known about the nature of factors acting upon nerve cells during their development. However, when international communications were restored at the end of the war, it appeared that American investigators (Detwiler, Piatt, Oppenheimer) had also been working on Mauthner's cells of teleosts and amphibians. The correlation of these lines of research carried on independently should lead to better insight into the phenomena concerned.

In order to determine whether intrinsic or extrinsic factors cause cells to acquire their specificity and in order to establish the stage when nerve cells become irreversibly fixed, the following series of experiments on Mauthner's cells was carried out.

The experiments were done in embryos of *Rana*, which is a form very suitable for experimental manipulation and is available in Italy during most of the year. The presumptive "Mauthner's zone" of early larvae in different stages of development was excised, and the fragments were either implanted in the body cavity of larvae of the same stage or explanted *in vitro*. The purpose of this technic was to isolate the presumptive zone from its normal environment, so that the intrinsic potentialities of the embryonic cells could express them-

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hand, when the fragments are larger, the appearance of the Mauthner's cell is much closer to normal. These large explants show a considerable degree of morphological organization, and often fragments removed as lateral halves restore the missing contralateral half, thus giving rise to nearly symmetrical explants.

The high regulatory capacity of the embryonic medulla appears also under other circumstances. In a series of experiments, which had been designed to prevent the crossing of Mauthner's axons, a flap of skin was grafted along the mid-line of the region of the future fourth ventricle. Each half thereafter developed into a whole medulla, with the result that these embryos had two medullas side by side. Each medulla, however, had only one Mauthner's cell Morphological regulation, therefore, does not imply full histological restitution.

All Mauthner's cells developed in isolation appear to undergo involutive changes 25–30 days after the explantation. These changes are probably due to functional inactivity. In some cases in which the presumptive Mauthner's zone was transplanted into the spinal cord the involutive phenomena appeared to be accelerated. I do not think that such involution should be ascribed to the heterotopic location to which the cell was grafted but rather to abnormal physiological activities. This process may be similar to the involution that Mauthner's cells normally undergo during metamorphosis, when they lose their function because of the degeneration of the tail (Larsell, 1934).

Mauthner's neurons have also been used (by Piatt, by Oppenheimer, and by myself) for investigations concerning the direction of growth and the crossing of nerve fibers. In the growth of a nerve fiber we must distinguish between a passive and an active aspect. The first is represented by the structural and ultra-structural organization of the substratum that acts as a directive influence for the growing fiber We could compare it to a tube through which a growing plant root passes. The second is of a tropic nature and would imply one or more sources of stimuli that attract the outgrowing fiber, comparable to a root that grows toward a source of moisture. We can imagine that both these factors coexist, comparable to a root reaching a source of moisture through a tube placed along its path. But, as roots, apart from their growing mechanism, keep their own morphological characteristics, so we might imagine that the nerve fibers also have intrinsic characters which they acquire at the moment of their determination.

The behavior of the processes in some of the experiments done

selves. After varying periods of time, both donor and fragment were studied histologically, with the following results:

1. When the fragment had been excised from a very young embryo (early gastrula), the donor contained two normal Mauthner's cells. This means that the embryo was able to replace the lost material through complete regulation. Conversely, the isolated fragment, which developed into a vesicle or a small solid ball of nerve cell, was devoid of Mauthner's cells.

2. In embryos operated in the late gastrula stage, the donor behaved as in the previous case, but in some instances a Mauthner's cell was also found in the explant.

3. *Finally, in all operations performed at later stages (neural tube to tail bud)*, Mauthner's cells developed only in the fragments. [illegible]

[illegible]

ing Mauthner's neurons. It should be mentioned that Mauthner's cells that developed in explants, although always identifiable, showed certain alterations in shape and size.

The obvious conclusion of these experiments is that Mauthner's cells are not yet determined in the early gastrula stage, that their determination proceeds during late gastrulation and has become irrevocably fixed after that stage. This indicates that the neuroblasts of highly differentiated neurons attain their definitive structure in two steps. Through the first step the neuroblasts attain a general differentiation to nerve cells with prolongations, neurofibrils, Nissl bodies, etc., while during the second stage more definite characters are impressed on the cell, such as form and number of processes, shape and size of the cell body, etc., that is, those characters which distinguish a Mauthner's cell from other types of neurons.

Although it is clear that Mauthner's cells possess intrinsic capacities for specific differentiation (see also Detwiler, Piatt, and Oppenheimer), it must be equally stressed that the elaboration of these potentialities is greatly influenced by external factors, such as the orientation and nature of the substratum, acting particularly upon the growth of the processes. This fact becomes evident when the presumptive Mauthner's zone is allowed to develop in explantation instead of in transplantation, for this technic permits us to control the size of the fragment.

We see then that when the explants are very small and the fibers consequently cannot grow sufficiently far for lack of space, the cell bodies appear smaller than usual and globular in shape. On the other

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with Mauthner's cells throws some light on these complicated relations.

1. *In the experiments in which the Mauthner's cells were allowed to develop in explants, as well as in those in which they were grafted to abnormal locations, the axons developed as short, coarse, club-shaped processes. It is thus evident that the growth capacity of the axon, although dependent upon the size of the territory, is still inherent in the cell.*

2. In those explants which restituted the contralateral half, producing a nearly symmetrical medulla, Mauthner's fiber always decussated, even if, after having reached the opposite side, its further growth was stopped for lack of space.

3. A definite difference in the behavior of axon and dendrites can be demonstrated by reversal of the Mauthner's zone (in the yolk-plug stage) along its anteroposterior axis. In these experiments one *observes that the axon, in spite of the abnormal orientation of the cell*, turns back at a sharp angle and reaches the spinal cord all the same. The dendrites, on the other hand, do not attain their normal destinations but branch out in the new abnormal location.

4. As I mentioned before, a group of experiments was undertaken in which a flap of skin was inserted along the mid-line of the presumptive medulla to prevent crossing of the fibers. The ectodermal tissue of the flap soon degenerates and becomes invaded by nerve cells. Mauthner's fibers always enter this region of irregular nerve tissue, but after a somewhat irregular course they bend caudad and reach their normal position along the median longitudinal fascicle, without decussating.

5. If a section of mesencephalon is grafted into the spinal cord, Mauthner's fibers are found to grow through this completely strange structure, without deviating from their course.

From all these facts the following conclusions can be drawn:

A. The cell possesses the capacity to grow an axon, the growth of the axon, in turn, reflecting on the size and shape of the cell body.

B. The growing fiber does not have an intrinsic tendency to follow a specific direction.

C. The directive influences acting upon the growing fiber are of two kinds: (1) structural organization of the substratum, acting as a mechanical guide as well as a thigmotropic stimulus and (2) tropic forces which manifest themselves during ontogenesis, the nature of which was not explored.

of peripheral tissue and the number of connecting nerve cells. It is not necessarily a simple matter of maintaining commensurate ratios between the two. Furthermore, there is evidence that in normal development more cells are actually present in various nervous centers than ultimately establish themselves as functional, definitive neurons. In these instances operational procedure is not a factor. There is no real increase or decrease of peripheral field in such cases, only the normal, proportional growth of the embryo. Peripheral regulation is apparently a fundamental factor in nervous development and not just an emergency response on the part of the organism.

The manner in which peripheral-central regulation is brought about is not clear. Both the proliferation and the differentiation of nerve cells are controlled to some extent, and there may be degeneration of the earlier-differentiated neurons. The effects of peripheral regulation are usually not manifested in the earliest stages of development. However, increased mitotic counts can be detected prior to the onset of actual structural growth of the related peripheral tissue. Further maturation of neuroblasts may be initiated by the "*inductive*" effect of neighboring cells which differentiate early and make the first contacts with the peripheral field. This process repeated could account for the regulation of the total number of mature cells finally innervating the structure. Such a hypothesis would not explain the earlier increase in mitotic rate. Finally, there is considerable evidence that the functional maturation of the peripheral structure plays no *significant part* in the achievement of the total cell number in related nerve centers.

Refinements in technic and analysis during more recent years have given us better insight into the problem of peripheral regulation of the central nervous system. Despite this fact, however, it is my opinion that experimental embryology has as yet found no real solution to the problem. We describe what we find, but, so far, we do not see the operational connection between the events. At present there are no experimental data which can be colligated into a working hypothesis.

The problems of intra-central connections and peripheral nerve patterns are no less obscure. Particularly is this true of the former. When a lateral half of the embryonic salamander mesencephalon is reversed end for end, many of the tracts and fiber pathways fail to develop normally and do not *grow* through the reversed mesencephalon to reach their proper nuclei. Some of the tracts do, however. The mesencephalic V root fibers frequently extend themselves through

DIFFERENTIATION AND GROWTH OF NERVE CELLS AND FIBERS

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THE study of the differentiation of nerve cells and of the growth and orientation of the cell body and its conducting processes is as old as the cell theory itself. It is not advisable in this short space to enumerate more than a few of the ramifications of this field of research and the questions which have arisen from the solution of the more basic problems. A great mass of experimental data is on record. I propose to discuss certain aspects of the general subject of differentiation and growth from the standpoint of present knowledge and future needs.

When the fore limb of a rat or salamander or the wing bud of a chick is extirpated, the primary sensory centers (brachial nerve ganglia) eventually contain fewer neurons than would have been the case had the limb remained intact. When an additional limb or wing bud is transplanted, the ganglia connected with it eventually contain more neurons than do the corresponding ganglia of unoperated controls. The primary motor areas of the cord are likewise reduced in number of motor cells (limb extirpation), or the number of motor cells is increased (additional limbs). This peripheral-central relationship is not limited to limb tissue but is manifested when any peripheral field is altered in amount Furthermore, the nerve centers affected do not necessarily have to be the ones normally innervating the region. This influence of the periphery over developing nerve centers has been described in all major vertebrate groups, including man. There is evidence also that the adult central nervous system and ganglia respond to quantitative changes in the periphery, and hence this effect is not limited to embryonic or fetal stages.

The nonnervous periphery helps in some way to regulate the number of neurons which connect with it. From one frame of reference this does not appear surprising. One would expect a larger area of skin to be innervated by a greater number of nerve cells, and the same could be said of muscle and motor neurons. On the other hand, there frequently is no linear correlation between the absolute volume

contact with the near-by limb primordium, and subsequent growth and differentiation of the limb tissues are responsible for the definitive nerve pattern. The simultaneous growth of nerves and limb tissue, however, makes analysis difficult. It also does not fit in with certain other experimental results.

If salamander fore limbs are caused to develop to larval stages without ever having possessed a nerve supply and if these aneurogenic limbs are then put in place of a fore limb in another previously unoperated animal of approximately the same age, the transplanted limb eventually receives its belated innervation from the proximal ends of the host's cut brachial plexus nerves. The resultant pattern of the regenerated nerves within the transplanted limb is not haphazard and random. An essentially normal pattern prevails. No distal degenerating nerves are present to guide the entering host fibers, and *relatively* long distances must be traversed through limb structures which have attained their essentially definitive outlines. Experiments [illegible] nerve patt[illegible] that norma[illegible] sarily conditioned by one invariable set of factors and, therefore, that our conception of peripheral nerve pattern is not so simple as it once appeared to be.

There is additional evidence that selective differentials exist between developing sensory and motor fibers. When the lumbosacral ganglia of young frog larvae are removed, only motor fibers reach the hind limb; when the motor region of the cord is ablated, only sensory nerves are available for limb innervation. Tadpole hind limbs with only motor fibers possess chiefly the motor portion of the nerve pattern; hind limbs having only sensory nerves form a typically cutaneous pattern. In view of these observations, one may well ask why we are so reluctant to credit selective differences among growing motor nerves themselves or between different sensory pathways. In this connection we are also reminded of the fact that lateral-line nerves will not innervate other types of end-organs and that general somatic afferent fibers do not innervate lateral-line organs. The problem of selective matching of nerve fiber and end-organ is not necessarily the same as that of nerve pattern per se, but in normal development one implies the other, and in any event it is a somewhat artificial separation. With regard to the pattern of peripheral nerves and the selective innervation of end-organs, we know something of what can happen and what cannot under certain experimental con-

the reversed tissue of the brain and enter their proper cranial nerve roots. The fasciculus solitarius in similar cases has also been observed to find its proper terminal field. Mauthner's cell, when transplanted to the region of the telencephalon in embryonic salamanders, will send out its axon, and the latter, after a circuitous route, usually ends up by occupying its normal position along the fasciculus longitudinalis medialis of the spinal cord. What is the explanation of these observed facts?

There can be no explanation until we know considerably more about the details of central nervous system development. Whether this needed information will eventually come from experimental procedure or from the classical approach of describing what is seen in the development of the normal animal remains to be discovered. It is true, however, that experimentation in the absence of fundamental knowledge concerning the relatedness of anatomical events in the growing central nervous system, particularly during later stages in development, is very likely to lead us nowhere. The brain is such a closely integrated mechanism, both anatomically and functionally, that individual fiber pathways are seldom thought of as possessing any independence of growth in themselves. The difficulties inherent in designing feasible experiments by the ordinary methods of experimental embryology have kept us pretty much in ignorance of the forces at work at later stages within the central nervous system.

Peripheral nerve patterns are more amenable to experimentation than intra-central ones. A salamander limb disk transplanted to the head or flank will secure its innervation from ectopic sources. The pattern of the nerves will depend upon whether the transplant is a fore limb or a hind limb. In other words, the source of nerve supply does not influence the pattern; the structural arrangement of the limb tissue itself is the deciding factor. Amphibian fore limbs innervated by only cranial nerves, by one brachial plexus nerve, or by flank spinal nerves alone all have essentially the same intra-limb pattern. Presumably this is true of other bodily structures as well, although the principle as stated has been derived chiefly from limb-transplantation experiments. This so-called "guiding" effect of the limb tissue upon the nerves has been thought of as demonstrating the nonselectivity of peripheral nerve pathways, the idea being that the nerve fiber itself has little choice in the matter. Although seldom stated in this unadorned, simple way, this appears to be the essence of the argument. It is strengthened by our conception of normal nerve end-organ development and growth. The earliest nerve fibers make

specialized neuron, and yet it will differentiate in small, isolated pieces of neural plate without benefit of structural organization or function. On the other hand, differentiation of a Mauthner's cell may be initiated much later in development through the influence of extrinsic, organizational factors, i.e., the ingrowth of ectopic VIII root fibers at the level of the V nerve. There is no reason to assume that these experimental data are contradictory. On the contrary, it illustrates a principle too often ignored in investigations of this sort, namely, that the developing organism may be capable of a wide variety of responses, depending on the conditions imposed upon it. We shall probably find that inherent factors initiating cell lineage are operative quite early in nervous development but that these factors are plastic and capable of new directions under later exigencies of the environment.

It is quite obvious that these problems are only a very few of the many which confront us and that the data presented are handpicked, so to speak. It is utterly impossible to present in so short a space anything even approaching a well-rounded picture of the differentiation and growth of the nervous system. The more fundamental aspects of neural growth deal with concepts and principles common to any study which seeks to discover why things happen as they do in developing living tissue. Since most of the problems are of a fundamental character, it should occasion no surprise that major solutions are so slow to appear As much is known about neural development as about any other field of embryology, perhaps more. The future prospects of neuroembryology are not, therefore, necessarily gloomy or disheartening I should like, however, to conclude this report with a few observations which I consider of [illegible] that they are my personal views and do not necessarily reflect the *outlook* of other members of the conference. For this reason they may be poorly conceived and somewhat exaggerated for emphasis; certainly, they are subject to re-evaluation at any time.

I believe that the traditional methods of experimental embryology are incapable of *giving us* the answers which we need. The technic of cutting out or grafting pieces of nervous tissue from young embryos is a relatively crude *procedure* when one reflects for a moment on the complex nature of the *questions* we are asking and the refinements in analysis we attempt to draw from the data. Neuroembry-

ditions. Several hypotheses have been suggested, and some fruitful work has been done. A single general principle governing directional nerve growth has, however, yet to be discovered.

Little is known concerning the factors responsible for the migration of neuroblasts within the central nervous system. It is an extremely important part of brain and cord development, and it is surprising that so little experimental investigation has been directed toward this problem. Instances of nerve-cell migration have been observed in tissue culture, and the neurological literature abounds with references to the migration and displacement of various nuclei and cell groups. The development of the cerebral cortex in ontogeny is only one of the more important examples. It would be interesting to know whether dispersal of cortical cells away from the central gray is brought about or implemented in any way by actual fiber connections from thalamic centers or whether peripheral migration of these neuroblasts is the result of purely intrinsic forces which have in some way become implanted in these cells during our evolutionary history. It is unfortunate that in this connection, as in many other phases of biological work, theories of phylogenetic development are confused with the development of the living, individual animal. If, for instance, it could be shown that the outward dispersal of the primordium hippocampi cells of amphibians, the first rudimentary beginning of the vertebrate cerebral cortex, was actually initiated by the ingrowth of ascending fiber tracts from lower centers, we would have some explanation for the development of this most important of all brain structures. As the situation stands today, we must extrapolate our answers from our knowledge of cerebral phylogeny, and, as such, they will be difficult to verify. Neurobiotaxis is the only theory of any standing on record which attempts to explain the migration of intra-central neuroblasts, and this principle itself was erected largely on the basis of phylogenetic interpretation.

The origin of divergent types of nerve cells has not been traced completely in many instances, and little, if anything at all, is known concerning the factors responsible for the initiation of divergence. It is known that Mauthner's cell will differentiate in small, isolated pieces taken from the early neural plate in the salamander embryo. This means that the cell is probably determined long before its first visible differentiation (about stage 37 in *Amblystoma* embryos). It is also possible to produce a supernumerary Mauthner's cell at the level of the V nerve roots by appropriate operational procedures on *Amblystoma* embryos as late as stage 27. Mauthner's cell is a highly

which corrects the rearrangement before the later processes of organization ensue. The approach, therefore, either falls short of, or exceeds, the requisite conditions demanded by the experiment.

It is obvious that this dilemma does not concern much of traditional neuroembryology, those questions involving very early neurogenetic problems. It does concern the experimental embryologist interested in the later neuroanatomical aspects of development. If structural differentiation and growth at the higher levels of development are to be studied, it will be necessary to employ additional methods and technics. Just what these added methods are to be I, for one, do not know. No doubt a team consisting of an experimental embryologist, a biophysicist, and a neurophysiologist could make some headway along the lines suggested. From some previous experiences, however, I should expect that keeping the neuroanatomical emphasis to the fore would constitute a problem in itself. The important thing is that the neuroembryologist should become more interested in neurology if the gap between the two fields is to be narrowed. Extirpation and transplantation methods are invaluable, but much of the data thus acquired have no bearing on later developments.

ology is still in the natural-history stage of its development; yet we are hoping for answers to questions which might well make a nuclear physicist shudder. The field is badly in need of a theoretical complexion, but we are pushing our limited data too far. This implies in no way that we should simply continue to pile up additional experimental data and facts and make no attempt to formulate principles and working hypotheses. Theoretical aspects should continually be brought to the fore because, without them, we are working in the dark. It does seem to me, though, that, as far as the differentiation of the central nervous system itself goes, we are reaching an impasse, assuming a continuation of the ordinary methods alone. The trouble is not actually so much a matter of theorizing on insufficient data as it is in spreading these data to encompass levels of central nervous system organization farther and farther removed from the embryologist's domain. This statement requires clarification.

With very few exceptions, experiments on the developing central nervous system fall into two chief categories: (1) actual manipulation of the neural axis itself or its presumptive areas to elucidate problems concerned primarily with the very early forces at work in neurogenesis and (2) alteration of peripheral nerves, sensory primordia, or nonnervous peripheral structures to observe the anatomical or functional effect upon the brain and spinal cord. Neither of these experimental approaches goes very far toward explaining the intricate structural organization of the definitive brain and cord. It is important to have knowledge of the forces which mold a neural tube from a flat plate of cells, but these are not the same forces which operate to determine origin, termination, and route of fiber tracts. The influence of the nonnervous periphery on central nervous organization is an important factor in neurogenesis, but the effect is essentially a quantitative one and only touches the problem of later organizational connections, that is to say, the qualitative anatomy of the brain and spinal cord. The point is this: Mechanical manipulation of the embryonic neural axis itself results in abnormalities of growth which so alter the normal configuration of the brain, both externally and internally, that it is often impossible to recognize, let alone analyze, the structures under investigation. I do not believe it is a question of good or bad technic. The difficulty is inherent in the operational methodology itself. Less radical operations are just as frequently at fault because of the great capacity for regulation, particularly in amphibian embryos, in the early neural axis,

regenerative capacities of different neuron types. It is interesting that the conference revealed that such aspects of constitutional diversity are now receiving increasing attention by those interested in their embryological origin. In the adult such diversity is exhibited not only in the astonishing morphological variety of nerve cells but also in cytological characteristics, such as staining reactions; in metabolism, as shown by varied reactions to anoxia, to drugs, and to neurotropic viruses; in the selective affinity of outgrowing axons for specific end-organs (Speidel); and in the varied reaction of nerve cells to axon interruption.

All these constitutional variations of nerve cells, which obviously must be associated with underlying chemical and metabolic differences, have scarcely been approached by biochemists, most of whom, of course, could hardly be aware of the existence of problems which have as yet been attacked only tentatively by the biologists themselves. Yet the mere existence of numerous criteria of diversity among neurons makes possible an attack from various angles.

In our own experience, one pathological criterion of diversity—the difference in susceptibility of neurons to poliomyelitis virus (1)—clearly meets a basic biological one, the diversity of metabolism of these neuron types. An approach to the problem of how these two phenomena are related is revealed by the finding of Dr. Howard A. Howe and the author that normally susceptible neurons can be made relatively resistant by section of their axons (2). In other studies we have shown that such section of axons leads to profound metabolic changes in the cell bodies during the time when axon regeneration is in progress (reviewed in Ref. 3) In studying the diversity of pathological response in relation to metabolic specificity, it is the hope that clues may be found that will help clarify both problems.

Axon regeneration itself is a greatly varying property of nerve cells, and one which offers another approach to the problem of the constitutional diversity of neurons Severance of the axon process of some nerve cells in the central nervous system results in irreversible changes, leading to cell dissolution in 1 or 2 weeks. Other central neurons respond by assuming a condition of persistent atrophy, without regrowth of the axon. Nerve cells whose axons lie in peripheral nerves, however, respond to amputation of a large cytoplasmic [illegible] [illegible]found but reversible structural and metabolic changes [illegible] [illegible]eration. In these cells the ad- [illegible] and the subsequent process of [illegible]

NEUROPATHOLOGY AND THE CONSTITUTIONAL DIVERSITY OF NEURONS

DAVID BODIAN

Poliomyelitis Research Center, Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland

WHEN one considers nervous tissue apart from its conducting function, it is plain that problems of development, maintenance, and regeneration are as complex and as challenging as those which more directly concern conduction of the nervous impulse. This statement is not intended to imply a separation of nervous conduction from other functions of nervous tissue but to introduce the thought that the latter functions have so far suffered undeservedly from relative neglect. The cell-to-cell transmission of the nervous impulse is a biological phenomenon of exceptional interest and importance and one which predominantly comes to mind when one thinks of the function of nervous tissue. The so-called "trophic" dependence of some nerve cells upon synaptic connections with other nerve cells for their maintenance and survival is an interneuronal relationship which has been given relatively little attention. Similarly, other specializations of the nerve cell, such as its inability to divide, its bizarre shape, its high content of cytoplasmic nucleoprotein, and its occasional ability to regenerate an amputated axon, have only begun to be investigated. Yet these properties must necessarily be adaptations related to the prime function of nerve cells and must be understood before a complete understanding of nervous conduction is possible. Moreover, the peculiarities of nerve cells and their supporting tissue, whether seen from the morphological, physiological, or metabolic level, represent the basis for understanding many problems of neuropathology. Although the clinical expression of neuropathological processes of many sorts is dependent on derangements of conducting mechanisms, by and large the etiological basis of these processes must be ap-

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e are, first, the

meaning of the great differences of susceptibility of nerve-cell varieties to injuries of many kinds and, second, the reason for the varying

regenerative capacities of different neuron types. It is interesting that the conference revealed that such aspects of constitutional diversity are now receiving increasing attention by those interested in their embryological origin. In the adult such diversity is exhibited not only in the astonishing morphological variety of nerve cells but also in cytological characteristics, such as staining reactions; in metabolism, as shown by varied reactions to anoxia, to drugs, and to neurotropic viruses; in the selective affinity of outgrowing axons for specific end-organs (Speidel); and in the varied reaction of nerve cells to axon interruption.

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an adult cell in which cell division does not occur. Moreover, the induced changes in levels of important chemical components and of enzymatic activity in the cell can be compared with normal levels and correlated with altered functions. It is interesting that all changes so far observed in the regenerating neuron, both morphological, physiological, and chemical, are in the direction of reversion toward the levels of the embryonic period (Flexner), with subsequent return to adult levels as regeneration is completed. Both the developing and the regenerating neurons appear to have relatively low levels of cytochrome oxidase activity, low levels of accumulated cytoplasmic nucleoprotein, high levels of phosphate turnover, and increased demands for protoplasmic synthesis.

Why nerve cells respond so differently to axon interruption is a problem which has scarcely been broached. Some central neurons die quickly, some persist in atrophied form without effective axon regeneration, and, of course, peripheral axons regenerate readily. Are these differences correlated with specific differences in morphology or in metabolism? In the instance of central neurons, is death or survival after axon section dependent on the absence or presence of proximal collateral axons or on differences in metabolism? Some insight into the causes of this varying capacity for axon regeneration may result from further studies of the process in cells which readily regenerate their severed axons.

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SPECTROSCOPIC STUDIES ON NERVE CELLS IN DEVELOPMENT, GROWTH, AND FUNCTION

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FOR all who had the advantage of participating in the conference on "Development, Growth, and Regeneration of the Nervous System," the seven days filled with discussions and the data given by the different specialists were a great impetus to carry on their own investigations under the influence of the new results reported. This conference certainly stressed the importance, for the active workers in a certain field, of being able to discuss the problems in the form of such a symposium.

Regarding the topic of the conference, a limited amount of data has been collected during recent years by the use of cytochemical and biophysical methods. These data can be used to consider the prob- [illegible] and chemical composition of the neurons during development and differentiation and under different functional conditions.

METHODS

The technical difficulties when studying the chemical composition and physical properties of the cellular elements of the central nervous system are obvious. For the following discussion I will comment upon the spectroscopic methods, which have been used in the quantitative determination of certain chemical groups and of the cell substance within single cells. A simple calculation of the amounts of substances which have to be determined shows the demands upon the methods. One μ^3 has a weight of 10^{-12} gm., supposing a specific weight of 1. Let us assume a nerve cell with a volume of 10,000 μ^3 and a weight of 10^{-8} gm. Detailed analysis of areas within this cell requires a method which permits of a determination of 10^{-13}–10^{-14} [illegible]

an adult cell in which cell division does not occur. Moreover, the induced changes in levels of important chemical components and of enzymatic activity in the cell can be compared with normal levels and correlated with altered functions. It is interesting that all changes so far observed in the regenerating neuron, both morphological, physiological, and chemical, are in the direction of reversion toward the levels of the embryonic period (Flexner), with subsequent return to adult levels as regeneration is completed. Both the developing and the regenerating neurons appear to have relatively low levels of cytochrome oxidase activity, low levels of accumulated cytoplasmic nucleoprotein, high levels of phosphate turnover, and increased demands for protoplasmic synthesis.

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3. Bodian, D. 1947. Nucleic acid in nerve-cell regeneration. Symp. Soc. Exper. Biol., No. 1: Nucleic acid.

graphed in a microcamera at a magnification of around four hundred, and the density on the photographic plate is determined photometrically. This method has proved to be of great value in evaluating quantitatively the difference in the total amount of cell substances in the nerve cells in different functional states.

As has been pointed out by various authors, the capacity of nerve cells to bind basic dye groups with great avidity is due to their content of nucleic acids. The staining reaction of the Nissl substance can thus be traced to these chemical substances. Especially regarding the nervous system, however, there have been attempts to try to evaluate by the eye the color intensity under the microscope and to refer

TABLE 1*

[illegible]

E_{260}	E_{280}	$E_{corr}260$	$E_{corr}280$	Q $E_{corr}(260/280)$	Per Cent NA
0 422	0 297	0 308	0 194	1 54	1 8
493	369	372	258	1 44	2 1
461	340	.340	227	1 50	1 4
472	343	352	224	1 57	2 0
430	297	316	194	1 63	1.5
474	327	356	222	1 60	0 9
.440	344	318	239	1 39	1 6
500	362	362	237	1 59	2 0
519	253	253	146	1 61	2 0
450	315	307	202	1 53	2 1
427	329	315	227	1 39	2 0
457	318	337	213	1 58	0 7
502	378	382	269	1 42	2.8
.394	300	278	194	1 43	0 8
452	341	330	219	1 51	2 1
472	305	285	201	1 42	2 0
447	317	320	204	1 57	1 4
407	295	293	193	1 53	1 2
333	379	405	260	1 56	2 2
455	304	312	198	1 59	1 5
449	329	324	217	1 49	2 6
416	304	326	208	1 56	1 7
424	301	304	193	1 58	1 6
422	297	293	187	1 59	2 1
312	390	384	206	1 39	2 2
459	366	368	259	1 42	1 4
455	329	328	216	1 52	1 9
442	301	302	193	1 58	1 6
440	315	318	215	1 49	1 5
0 473	0 326	0 342	0 220	1 55	1 6
		m=0 327	m=0 216	M= 1 52±0 01	m=1 7

* Both the primary values for extinction at 2,600 and 2,800 A and the same values corrected for unspecific losses of light are shown. The relation between the nucleic acids and the protein substances can be calculated from the quotient for the corrected extinctions at 2,600 and 2,800 A, E_{cor} (260/280)

nucleic acids have an absorption maximum at 2,600 A, depending upon the content of purine and pyrimidine bases. The absorption is identical for the two types of nucleic acids—ribose and desoxyribose nucleic acids.

The type of nucleic acid present is determined by the use of a ribonuclease free from proteolytic activity, by means of absorption spectra before and after the digestion. The Feulgen reaction is specific for desoxyribose nucleic acids.

In an article about the quantitative determination of nucleic acids by spectroscopic measurements, Commoner and Lipkin (1949) have pointed out from a purely theoretical point of view the possibility that the extinction around 0.3 μ may be due to the orientation of the nucleic acid molecules. According to these authors, the occurrence of maxima at 0.3 μ suggests that the orientation effect has influenced the values obtained. As Pollister and Swift (1949) point out, the data from fixed nerve cells do not support this view if due allowance is made for the nonspecific light loss. As is also seen from Tables 1 and 2, the values are grouped fairly symmetrically about the mode. As Pollister and Swift stress, absorption data from cells, if properly analyzed, all comply with the Lambert-Beer law.

Also the specific absorption due to certain amino acids in the cell protein around 2,800 A can be determined in localized parts of cells.

In a series of investigations the importance of the nucleic acids in the most primary of all biological processes—the reproduction of genes and cellular growth—has been stressed. The reproduction of gene elements during cell division seems to be mediated by desoxyribose nucleic acids (Caspersson, 1940*a;* Caspersson and Schultz, 1938). In the production of cellular proteins during growth and in functions which chiefly concern the cytoplasm, pentose nucleic acids are rapidly broken down and regenerated. This is the case which will be considered in the following discussion on the nerve cells.

Another quantitative method is the determination of the total amount of cell substance by mass determinations by x-rays according to Engström and Lindström (1949). The method is based on the fact that the absorption of suitably selected continuous x-radiation is proportional to the total amount of substance per surface unit in the cytological section. The wave length used is approximately 4–10 A. No element present in large quantities in the tissue has any disturbing absorption jump in this range. The cell to be investigated is photographed on a Lippman film, together with a reference system consisting of a wedge of collodion foils. The picture of the cell is photo-

of color, it was difficult to say from case to case whether or not the section belonged to an animal with an increased amount of nucleic acids in the nerve cells. It was found that light changes in the staining procedure and duration of staining and of dehydration easily changed the whole result and made the difference between the two types of cells indistinct. If the staining procedure was kept under identical conditions (all slides stained simultaneously), the results were more uniform, and the difference between the cells containing more or less nucleic acids was easier to see.

It is worth stressing that only the results of spectroscopic analyses within the ultraviolet and by means of x-rays can give accurate estimates of differences in content of nucleic acids or of any other substances possible to determine.

DEVELOPMENT

From a quantitative point of view, the differentiation of neurons affords many interesting details. If the apolar neuroblast is taken as a starting point, the production of cell substance, i.e., mainly cell proteins, is the largest for any one single cell unit in the organism. The increase of cell proteins amounts to more than two thousand times (estimated for the cytoplasm) during development from an apolar neuroblast to a young anterior horn cell. Assuming a cell protein containing 5 per cent tyrosine and 1.5 per cent tryptophane, the computation from the ratio of $E(260/280)$ shows that the ratio of protein to nucleic acid in the cytoplasm changes from 4:1 to 10:1. This means that there occurs a considerable increase of protein in comparison with the nucleic acids during the differentiation and development of the neuroblast. This is all the more important, as the increase in the nucleic acids in the cytoplasm of the neuroblasts is high in itself.

The volume of the neurite exceeds many times that of the cytoplasm. A conservative estimate shows that the increase in total cell substance during the development from neuroblast to young anterior horn cell amounts to more than two hundred thousand times.

INTRA-CELLULAR DIFFERENTIATION

Regarding regeneration and specificity of neurons and metabolic processes [illegible] would like to call attention [illegible] the nerve cell, especially t[illegible] the nucleus. This includes a biochemical differentiation which is characteristic of the nerve cell and parallels the differentia-

differences in the color intensity to differences in content of nucleic acids in the cells. Tables 1 and 2 show the danger of such a procedure. In Table 1 there are listed the primary figures of the extinction at 2,600 and 2,800 A in areas of anterior horn cells belonging to the ventrolateral group from the cervical intumescence in the rabbit. After correction for unspecific light losses, the determination of the nucleic acids showed, on an average, a content of 1.7 per cent. The data in Table 2 were obtained from animals in which the nucleic acid content was almost double, owing to treatment of the animals by a chemical substance, malononitrile (Hydén and Hartelius, 1948). The average concentration of nucleic acids was found to lie at 3.1 per cent.

Sections of the spinal cord from the two groups of animals were stained with toluidine blue under identical conditions and examined closely under the microscope. Judging the cytoplasm by the intensity

TABLE 2*

DATA FROM 28 COMPLETE ABSORPTION SPECTRA TAKEN AT POINTS IN ANTERIOR HORN CELLS (VENTROLATERAL NUCLEUS) FROM RABBITS TREATED WITH 4 MG. $CH_2(CN)_2$ *PER KILOGRAM BODY WEIGHT AND KILLED 1 HOUR AFTER INJECTION*

E260	E280	E_{corr}260	E_{corr}280	Q E_{corr}(260/280)	Per Cent NA
0 865	0 679	0 633	0.534	1.18	3 0
.892	805	692	603	1 14	2.9
.907	739	643	530	1 21	3.4
.830	.701	.622	.528	1.17	3 0
.911	777	.704	598	1 17	3 3
.858	.712	652	533	1 22	3.4
.739	.567	.531	393	1 35	3 4
930	769	720	591	1 21	3.9
.890	.715	689	562	1 22	4 1
.879	781	672	603	1 11	2 8
820	686	620	521	1 19	3 0
.859	720	643	543	1 19	3 4
.905	781	703	611	1 15	2 9
914	810	711	635	1 11	2 7
831	698	631	532	1 18	3 1
.930	.837	730	662	1 10	2.8
.889	742	682	571	1 19	3 4
.881	.705	673	567	1 18	3 2
.890	758	692	586	1 16	3 0
.790	715	584	506	1 15	3 1
.847	672	609	500	1 21	3 3
858	698	641	518	1 23	3 0
800	680	592	507	1 16	2 9
.825	785	615	578	1 06	2 0
.840	768	623	566	1 10	2 8
.902	.790	702	627	1 12	3 0
.900	.791	691	612	1 12	3.5
0 859	0 692	0 628	0 520	1 20	3.2
		m=0 654	m=0 558	M= 1 17±0 01	m=3 1

* Values shown as in Table 1.

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0.839	0 692	0 623	0 520	1 20	3 2
		m=0 654	m=0 559	M= 1 17±0 01	m=3 1

* Values shown as in Table 1.

different conditions, thus showing a *specificity* in reaction capacity. The main bulk of the nucleolus, containing ribose nucleic acids, thus increases in spinal ganglion cells of *Lophius piscatorius* during the production of cytoplasmic nucleic acids. It also increases in the first stage of poliomyelitis infection in motor root cells. The nucleo-

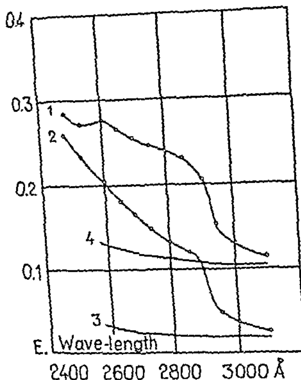


FIG 1—Absorption spectra from the chromcenter in a spinal ganglion cell nucleus from a rabbit (curve 1) and from a point in the adjacent nuclear substance (curve 2) Curve 1 shows a nucleotide band and a marked protein band with a tyrosine maximum shifted toward long wave lengths Curve 2 shows merely a faint band at 2,850 A Curves 3 and 4 indicate the loss of light by scattering

lus-associated chromatin, containing desoxyribose nucleic acids, is usually restricted to a limited number of small particles 0.5–2 μ in diameter. In the nerve cells of the ganglion cochleare, however, they are larger, in some cells filling nearly the whole area of the nucleolus (Hamberger and Hydén, 1945). The chromocenter substance, containing basic proteins and, to a small degree, pentose nucleic acids, has so far been investigated only in anterior horn cells and spinal ganglion cells. It seems to be lacking in small nerve cells. This substance increases in sensory nerve cells on adequate stimulation

tion of the cytoplasm. In the bipolar neuroblasts the first traces of the acidophilic nucleolar mass are seen in a chromocenter area, which undergoes dissolution. The main bulk of the nucleolus is distinguished by proteins with a basic character and also by ribose nucleic acid. This chemical composition explains its amphophilic character. At the periphery of the nucleolus, however, several particles containing desoxyribose nucleic acid are present. They have been designated as "nucleolus-associated chromatin." Rabinowitch (1949) has shown some activity of acid phosphatase in these particles.

For many reasons the nucleolus has been interpreted as derived from heterochromatin (Caspersson). Then nucleolus-associated chromatin can be looked upon as a residue of ontogeny which is characteristic of the nucleolus.

The nerve cell exhibits further differentiation of the nucleolar apparatus and parts of the nucleus associated with it. This has been analyzed by cytochemical methods (Hydén). In the vicinity of the nucleolus there is an optically denser nuclear area lacking distinct structure. In staining, it proves to be more acidophilic than is the remainder of the nucleus. Cytochemically it is characterized by high absorption maxima around 2,850 A, indicating a high concentration of protein. This structure takes acid dye groups with avidity. It also contains smaller amounts of pentose nucleic acids. Its chemical composition reminds one of that of the nucleolus and also of the heterochromatic areas of chromosomes from the salivary glands of *Drosophila*. In the nuclei of nerve cells of the male rabbit, which has an X- and a Y-chromosome, this substance was found to be more abundant than in the female. This agrees well with the fact that the Y-chromosome in the male is, in large part, heterochromatic. This area in the nerve cell was interpreted as a greatly increased heterochromatic chromocenter area belonging to the nucleolar apparatus (Hydén, 1943).

As it has a specific chemical composition and reacts in a specific way with certain viruses (see below), the picture of the nucleolar apparatus in the nerve cell is quite complex. It consists of three structurally and chemically different parts: (1) the main bulk of the nucleolus, containing protein of distinctly basic character and ribose nucleic acids; (2) the nucleolus-associated chromatin, containing desoxyribose nucleic acids; and (3) the heterochromatic chromocenter area containing distinctly basic protein and small amounts of pentose nucleic acids. These three components vary in amount in different types of nerve cells. They also react independently under

Nor do the scarce data available support the assumption that the yellow pigment in the nerve cells is a "wear-and-tear" substance. The pigment contains a lipoid part which is soluble in organic solvents, and another part with yellow color stainable with basic dyes at a pH > 5.5. Absorption spectra and x-ray investigations have given the following results (Hydén and Lindström, 1950): Absorption

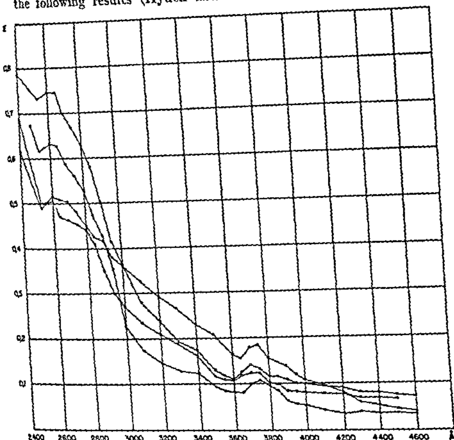


FIG. 2.—Absorption spectra taken between 2,400 and 4,600 A in areas of anterior horn cells containing yellow pigment from human spinal cord

spectra show a maximum at 2,600 A and another absorption band at 3,700–3,750 A (Fig. 2). This being the characteristic spectrum of flavins, the test of Warburg and Christian (1932) was applied. The reaction did not give positive results, nor was it possible to remove the absorbing part of the yellow pigment by ribonuclease. Absorption spectra taken before and after digestion were the same. On the other hand, the ribose nucleic acids in the remainder of the cytoplasm were removed. X-ray investigations showed that the area containing yel-

(Hamberger and Hydén, 1949 *a*, *b*) and in anterior horn cells in ischemia (Hochberg and Hydén, 1949), as well as in the first stage of poliomyelitis infection (Hydén, 1947).

ADULT NERVE CELLS

Large amounts of ribose nucleic acids in the cytoplasm are typical of all big nerve cells, even in the adult stage. These acids seem to be associated with proteins in the form of nucleoproteins. The maintenance of a certain concentration of nucleic acids in the cytoplasm of the nerve cell or their rapid regeneration after depletion seems necessary for the function of the neuron. The demand for a constant production of nucleoproteins seems to be great. This explains the extreme development of the nucleolus in the nerve cell, for the nucleolus plays an important role as mediating center in the production of the nucleic acids of the cytoplasm. This process has been observed and analyzed quantitatively in the spinal ganglion cells from *Lophius piscatorius*.

It is fair to state that nerve cells are decidedly embryonic in character. It is often said that the nerve cells in retrograde reaction resume distinctly embryonic properties, but they really have never lost them.

Is the nerve cell further differentiated? If one restricts differentiation to a structural and chemical change in a cell with maintenance or development of a specific function, there is a further differentiation of several types of big nerve cells, such as motor root cells, pyramidal cells, sensory and sympathetic ganglion cells. At the age of seven to eight years in man the first traces of a yellow pigment (lipochrom, lipofuscin) can already be observed. The substance occurs earlier in the nerve cells of the spinal cord and in the medulla oblongata (Mühlmann, 1901). Between the ages of forty and seventy years, it is present in nearly all the nerve cells in this area. The yellow pigment is of great interest because it is present in great amounts in the cytoplasm of more and more nerve cells as age increases, with no obvious relation to pathological changes. After the age of forty-five in man, most of the big motor nerve cells of the spinal cord contain more or less yellow pigment in their cytoplasm. Sometimes the main part of the cytoplasm is occupied by the yellow pigment, and very small amounts of nucleic acids can be observed. If the pigment, as has frequently been assumed, represents slags impairing the function of the cell, such impairment of function ought to be demonstrable. No correlation between function and amount of the yellow pigment in motor nerve cells has, however, been observed.

Nor do the scarce data available support the assumption that the yellow pigment in the nerve cells is a "wear-and-tear" substance. The pigment contains a lipoid part which is soluble in organic solvents, and another part with yellow color stainable with basic dyes at a pH > 5 5. Absorption spectra and x-ray investigations have given the following results (Hydén and Lindstrom, 1950): Absorption

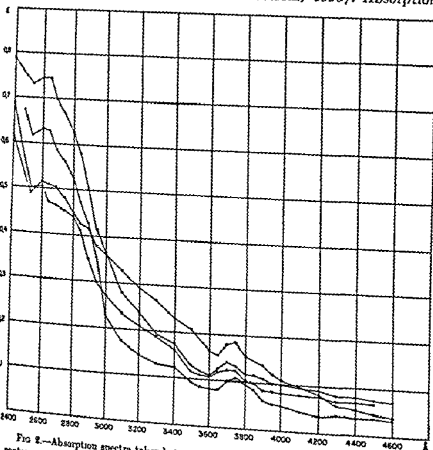


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low pigment had around 50 per cent more dry substance than did corresponding areas containing nucleic acids and proteins. An example from the analyses is given in Table 3.

Although the data on the composition of the yellow pigment are scarce, it is evident that a partial chemical reorganization of many of the nerve cells takes place with increasing age. The amount of ribose nucleic acids decreases in the cytoplasm, and their place is occupied by the yellow pigment with its characteristic absorption spectrum. There is, however, no commensurate reduction in motor function with increasing age. The experiments reported here thus speak against the interpretation of the yellow pigment as a slag product imperiling cell function. This must be kept in mind when dealing with the regeneration of the neurons. If it should become possible to analyze the composition of the yellow pigment more closely, the next problem would be to investigate whether or not the yellow pigment is consumed and regenerated by intra-cellular synthesis in the nerve cells in connection with the function of the neuron, as are the nucleoproteins.

TABLE 3

MASS DETERMINATIONS BY X-RAY MICRORADIOGRAPHY

Cytoplasm Containing	Weight (In $\mu g/\mu^3$)	Thickness (In μ)	Per Cent Dry Substance
Ribose nucleic acids and proteins	4.2×10^{-4}	11.3	37
Yellow pigment. . .	5.5×10^{-4}	9.8	56

REGENERATION OF NUCLEOPROTEINS IN THE NERVE CELLS UNDER PHYSIOLOGICAL CONDITIONS

With the cytochemical and biophysical methods available, one of the most important tasks is to study the relation between structure, chemical composition, and function of the nerve cells. In a series of investigations it has been demonstrated that nucleoproteins are consumed in the cytoplasm of the nerve cell in response to increased functional demands. This phenomenon has been shown in a variety of nerve cells, motor as well as sensory. Varying rates in the production of the nucleoproteins must be expected, depending on the functional demands, and the nucleoprotein-producing system of the cell must be able to cover a wide range.

Some experiments will be briefly quoted, in which the nucleoprotein formation in nerve cells was studied under the following condi-

tions: (1) resting condition with maintenance of function; (2) increased activity; (3) fatigue; and (4) neuronal damage with abolished function.

The experiments were carried out on rabbits and *guinea pigs on* the nerve cells belonging to the VIII nerve, the first neurons in the vestibular ganglion, and one group of the second neurons, the *nerve* cells in Deiters' nucleus. The animals were submitted to adequate stimu-

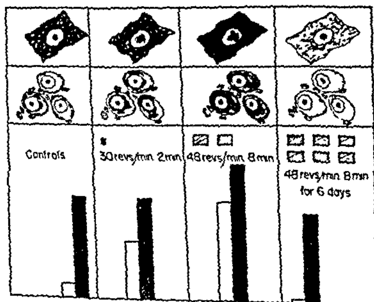


FIG 3—Diagram illustrating the correlation between

lation by means of rotation. The functional response and the state of the animal were tested by vestibular reflexes (Hamberger and Hydén, 1949 a b). Inte

teen 30-s

rection a

period. The animals were killed 1 hour after the last rotation. Intense stimulation causing fatigue was produced by such rotations repeated every day for 5 days. After stimulation for 8 minutes, the spectroscopic measurements showed an increase in the content of nucleoproteins in the cytoplasm of the vestibular ganglion cells as well as of the big nerve cells belonging to

low pigment had around 50 per cent more dry substance than did corresponding areas containing nucleic acids and proteins. An example from the analyses is given in Table 3.

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TABLE 3

MASS DETERMINATIONS BY X-RAY MICRORADIOGRAPHY

Cytoplasm Containing	Weight (In µg μ^{2})	Thickness (In µ)	Per Cent Dry Substance
Ribose nucleic acids and proteins	4.8×10^{-8}	11.8	37
Yellow pigment	5.5×10^{-8}	9.8	56

REGENERATION OF NUCLEOPROTEINS IN THE NERVE CELLS UNDER PHYSIOLOGICAL CONDITIONS

With the cytochemical and biophysical methods available, one of the most important tasks is to study the relation between structure, chemical composition, and function of the nerve cells. In a series of investigations it has been demonstrated that nucleoproteins are consumed in the cytoplasm of the nerve cell in response to increased functional demands. This phenomenon has been shown in a variety of nerve cells, motor as well as sensory. Varying rates in the production of the nucleoproteins must be expected, depending on the functional demands, and the nucleoprotein-producing system of the cell must be able to cover a wide range.

Some experiments will be briefly quoted, in which the nucleoprotein formation in nerve cells was studied under the following condi-

nucleolus was found to be deranged. In the nerve cells belonging to Deiters' nucleus the analyses showed a substantial decrease in the content of the nucleoproteins (see Fig. 5). This phenomenon could be correlated with the cessation of the vestibular function.

bound to the

idied the Purkinje cells in the cerebellum, the nerve cells belonging to nucleus XII, and the nerve cells in the inferior olive but found no corresponding

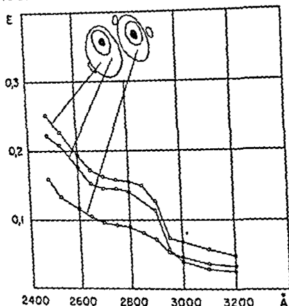


Fig 4—Absorption spectra taken at point in the cytoplasm of the ganglion cells from guinea pigs treated with streptomycin The schematic drawing shows the measuring points. The curves show only weak absorption maxima at 2,800 A No nucleic acid band can be observed

changes. The action of Streptomycin was interpreted as an inhibition of the intra-cellular reproduction of nucleic acids. It could not be established that the nerve cells belonging to the cochlear nerve were also affected Further experiments may show whether this is the case when the animals are permitted to live longer after the administration of the drug.

Another example is given by the results of infection of suitable nerve cells by viruses. As was shown by Bodian, only certain groups of nerve cells

Deiters' nucleus. Especially significant was the reaction of the *chromocenter area in the nucleus of the Deiters' cells. The increased* production of nucleoproteins was interpreted as a physiological answer of the nerve cells to increased functional demands. It seems that, in principle, such transneuronal chemical changes can be used to trace neuronal pathways.

On repeated stimulation for 6 days the duration of postrotatory nystagmus decreased. This was interpreted as an indication of fatigue of the vestibular apparatus, whose function was, nevertheless, unaffected. The total amount of ribose nucleic acids was found to be decreased in the vestibular ganglion cells of the rotated animals, as compared with those of the controls. This seems to indicate that the *system for the nucleoprotein production in the nerve cells is unable* to compensate for the consumption resulting from the increased functional demands. The same result was found in Deiters' nucleus, *although with less extensive chemical changes. The diagram in Figure* 3 illustrates the correlation between the cytochemical changes in the nerve cells of the vestibular ganglion and those of Deiters' nucleus in connection with adequate vestibular stimulation.

BIOCHEMICAL SPECIFICITY

Structural differences are often referred to as indicating functional differences. With the cytochemical data already obtained on nerve cells, it seems logical to try to attack the problem of biochemical specificity. Can nerve cells belonging to a certain neuronal pathway be shown to react specifically to a given stimulus or when subjected to damage? I should like to mention two examples. The first is a continuation of the experiments carried out on the vestibular nerve cells after adequate stimulation (Floberg, Hamberger, and Hydén, 1949).

From the beginning of its use, it was apparent that Streptomycin has a specific toxic effect on the vestibular nerve. It can give rise to symptoms of inhibition from the vestibular nerve, which also may result in complete cessation of its function. This raises the question of whether the drug acts on the peripheral or the central parts of the vestibular nerve. Our experiments were carried out on guinea pigs with doses of Streptomycin which caused symptoms of vestibular inhibition. Such a state was observed in the animals after 6–27 days, during which they had received intra-muscularly 75 mg. of Streptomycin per day. In the *animals showing loss of vestibular* function the vestibular ganglion cells were found to be completely devoid of nucleic acids in the cytoplasm (see Fig. 4). Also the structure of the

If animals are infected intra-cerebrally with rabies of the "fixe" type or the "street" type, the ribose nucleic acids decrease in the cytoplasm of the nerve cells affected when the clinical symptoms are pronounced. Concurrently with these chemical changes there is an increase in the number and size of the nucleolus-associated chromatin bodies containing desoxyribose nucleic acids. These changes are progressive and lead to amounts of desoxyribose nucleic acids such as are never found in nerve cells under physiological conditions. They may fill the whole nucleus. The particle size of these viruses is considerable, ranging from 150 to 200 mμ.

TABLE 4

NUCLEIC ACID AND VIRUS PARTICLE SIZE

Virus	Particle Size (Mμ)	Nucleic Acids Observed in Infected Cells
	Group I	
Rabies	150–200	Desoxyribose nucleic acids
Neurovaccinia	150–200	Desoxyribose nucleic acids
	Group II	
Poliomyelitis	15–25	Ribose nucleic acids
Louping ill	15–25	Ribose nucleic acids

In contrast with these findings are observations on nerve cells after infection with louping ill or poliomyelitis. Both these viruses have a small particle size, 15–25 mμ. In the beginning the same changes occur, namely, decrease and depletion of nucleic acids in the cytoplasm. In the nucleus, however, the chromocenter area in the vicinity of the nucleolus reacts. This substance increases, and spectroscopic measurements show an increased content of pentose nucleic acids and protein with a distinctly basic character. According to these findings, the viruses have been divided into two groups, depending on particle size and the type of nucleic acid found in the nucleus of the nerve cell after infection.

The viruses seem to act as parasites in the nucleoprotein-forming system of the cell (Caspersson and Hydén, 1945). The ribose nucleic acid appears to characterize the virus species having the lowest degree of organization. More complicated viruses, which demand a special mechanism to divide the different hereditary groups equally

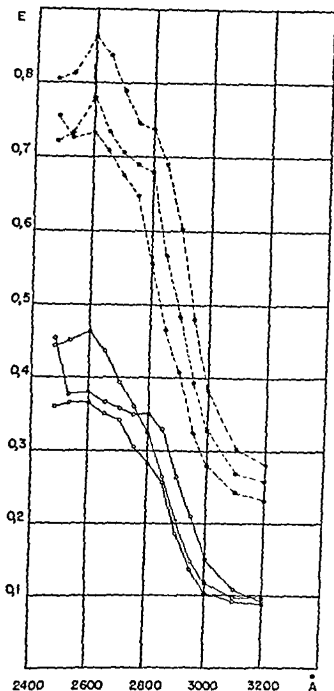


Fig 5.—A group of absorption spectra (*unbroken curves*) taken at point in the cytoplasm [illegible] regards their cytology in the ultraviolet. The absorption spectra are in agreement with those taken at corresponding measuring points in the control cell.

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in reproduction, seem to be characterized by desoxyribose nucleic acid. Striking in these examples are the sites of attack on the different parts of the nucleolar apparatus. The difference is quite clear and may be interpreted as reflecting a biochemical differentiation.

REGENERATION OF THE NEURONS

During development, there is an immense production of cell substance, including nucleic acids, in the neurons. In regeneration after the cutting of the axon, there must be nearly as high a demand on production of cell protein as during development. Assuming a spinal ganglion cell of average size, the volume of the regenerated axon can easily be computed to around 10,000,000 μ^3. This means a production of cell substance which is about a thousand times that of the cytoplasm.

During recent years attention has been concentrated upon the migration of substance in the axon, with strong evidence shown in the experiments of Weiss. One crucial point in this problem seems to be to correlate the total amount of substance produced in the nerve cell with the amount of the substance of the axon during the outgrowth.

In the cytoplasm a decrease of the nucleic acids after the cutting of the axon has been established (Hydén, 1942, 1943; Gersh and Bodian, 1943), which reaches its maximum around 15 days after transection. One possible technical approach seems to be the determination of the total amount of substance by means of x-ray spectroscopy.

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ther work (2) showed that succinic dehydrogenase begins to increase rapidly in activity a few days after this period.

The fetal pig is an inadequate experimental animal for a broad approach to this problem, in part because of its inaccessibility to physiological experimentation; and we have consequently used the guinea pig for our more recent studies. Let me first present the findings of V. B. Peters (3) on the morphogenesis of the nerve cells of the frontal cortex. The period from the forty-first to the forty-fifth days of gestation (term, 66 days) is critical in the development of this part of the cortex. Prior to this period, the cytoplasm of the neuroblasts shows a dustlike basophilia. Clumps of basophilic material, the Nissl bodies, are first seen in relatively few cells and in small quantity at a gestation age of 41 days. A remarkable increase occurs during the next 4 days and is followed by a relatively slight increase during the remainder of gestation and during postnatal life. It is during this same period, from the forty-first to the forty-fifth days of gestation, that there is a striking increase in the number and size of the cell processes. With the phase-contrast microscope it can be seen that there are relatively few processes at 41 days and that these are, for the most part, short and filamentous. By comparison, the cortex at 45 days has many well-defined, relatively long and stout processes; the change is a very striking one. Subsequent development of the processes involves continued increase in length and width. In addition, there is a change in refractive index which first becomes evident at 45 days, when certain of the processes show, for the first time, a higher refractive index, characteristic of the mature fiber, than do others Is this change in refractive index due to the appearance of neurofibrils or of the neural tubules of De Robertis and Schmitt? And, finally, using the methods of Chalkley for quantitative morphologic analysis (4, 5), Peters has found that, around the forty-third day of gestation, the nuclear volume of the average nerve cell reaches a value which, on the basis of her measurements, is indistinguishable from that throughout the remainder of gestation and that of the adult. Might this mean that the nucleus reaches maturity prior to the onset of those fundamental changes in the cytoplasm that are evidenced by the rapid accumulation of Nissl bodies and the intense elaboration of processes?

Although much time and effort have gone into the study of biochemical events during differentiation, the field is so broad that we have made no more than a firm and promising beginning. I have mentioned that in the pig cytological differentiation can be correlated

THE CYTOLOGICAL, BIOCHEMICAL, AND PHYSIOLOGICAL DIFFERENTIATION OF THE NEUROBLAST

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FROM the many broad problems relating to the development of the nervous sytem brought up for discussion at the conference by Dr. Weiss, those of us at the Carnegie Institution have chosen to confine our investigative interest to the differentiation of the neuroblast. I shall present briefly our viewpoint and findings and shall relate some of these findings to others presented at the conference.

In our present state of knowledge an essential endeavor is to describe the cytological, biochemical, and physiological changes which occur during maturation of the nerve cell and to relate these changes temporally to one another. A classical criterion for establishing the time of differentiation of the neuroblast into the neuron has been the appearance of clumps of basophilic material, the Nissl bodies, in the cytoplasm of the cell. The question immediately arises: If the appearance of Nissl bodies signals the differentiation of the neuroblast into the neuron, do other fundamental changes occur within the nerve cell at the same time? This question automatically suggests a series of investigations Those depending upon microscopic methods will be concerned with morphological changes in the nucleus, nucleolus, perikaryon, and cell processes. Those of a chemical nature will be concerned in part with the activities of essential enzymes and with the sources of energy available to the cell at various stages of its development. And, finally, one will use whatever methods are available for judging the functional status of the cell.

My introduction to this field came through a collaborative study with J. B. Flexner and W. L. Straus, Jr , on the developing cerebral cortex of the fetal pig (1). We found that there is a critical period, about halfway through gestation, when there is an abrupt increase in the quantity of Nissl substance, a rapid increase in size of the nerve cells, and a sudden rise in the activity of cytochrome C. Fur-

Kavaler (12) in an investigation, as yet incomplete, of the onset of peripheral response following cortical stimulation have demonstrated that all the peripheral responses elicited in a series of adults can be obtained in a fetus approximately 55 days old. Thus there is evidence that the axons of the cortical nerve cells reach their destination in less than 10 days after active sprouting is first observed. A measure of the rapidity of growth of these axons can be obtained from these observations. Those which end around the cells of origin of the motor nerves of the hind limbs grow a distance of 6 or 7 cm. in approximately 10 days.

We take the series of concomitant cytological, biochemical, and physiological changes which I have noted to signal the differentiation of the neuroblast into the neuron. Hydén (13) has described another important change which appears to fit this pattern. Using the ultraviolet microscope, he found that the nucleolus can be seen for the first time when the neuroblast is actively synthesizing protein and there is a simultaneous increase in cytoplasmic nucleoprotein. These findings, together with similar ones on damaged adult neurons in process of reconstitution, led to the highly stimulating view that the nucleolus and the nucleic acids of the cytoplasm are responsible for the formation of the protein of nerve.

All these studies on the maturation of the nerve cell appear to have an intimate relationship to the problem of nerve regeneration. It is a tribute to the insight of Van Gehuchten that in 1899, as a result of studies of Nissl preparations by himself and his student, Van Biervliet (14), he inferred that the chromatolytic, regenerating nerve cell returns to the embryonic status. Recent studies of enzyme activities in regenerating neurons fit this thesis Howe and Mellors (15) found that the activity of cytochrome oxidase is reduced in the chromatolytic cell; Bodian and Mellors (16), that the activity of acid phosphatase is increased; Howe and Flexner (17), that the activity of succinic dehydrogenase is decreased. These are the changes which would b[illegible]

[illegible] in view of the fact that both the regenerating and the immature nerve cell have a common function, the synthesis of neuroplasm.

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with an increase in the activity of cytochrome C, followed in a few days by an increase in the activity of succinic dehydrogenase. In the guinea pig, Belknap (6) has found the same relationship with respect to succinic dehydrogenase. J. B. Flexner, in her continuing study of the energy relationships during growth and differentiation, has found that the enzyme adenylpyrophosphatase, believed to make energy available to the cell from energy-rich adenosine triphosphate, has a low activity up to the forty-second day of gestation and then begins rapidly to increase (7). It is clear, consequently, that cytological changes which include the appearance of Nissl substance, growth of the nucleus to its final volume, and rapid sprouting of processes are accompanied by demonstrable changes in enzyme activities.

What can be said of the functional differentiation of the cortical nerve cells? This problem has been approached in three ways: by recording electrical potentials from the cortex, by observing peripheral response to electrical stimulation of the cortex, and by studying the behavior of the fetus.

In a study which is of great interest to us, Jasper, Bridgman, and Carmichael (8) found that they were first able to record electrical activity from the cortex of the fetal guinea pig at the forty-eighth day of gestation. Tyler, Gallant, and I (9) have confirmed these results, with the addition that we have recorded activity as early as the forty-sixth day, the end of the period which has been shown to be critical for cytological and biochemical differentiation. Efforts are now being made to determine whether these potentials are partly or wholly cortical in origin. In her biochemical studies, J. B. Flexner (10) made an observation which may give considerable insight into the events responsible for the onset of electrical activity. Up to a gestation age of 41 days, chloride and sodium are distributed in the same volume of cortex; both are apparently confined almost completely to the extra-cellular phase. At the forty-sixth day and thereafter, sodium is distributed in a considerably larger volume than chloride. It is tempting to ascribe this increase in permeability to sodium to the nerve cell and to suppose that the onset of cortical electrical activity is related to the appearance of a selective permeability to sodium, as has been hypothesized by Hodgkin and Katz (11) to account for the action potential or peripheral nerve.

Jasper, Bridgman, and Carmichael (8) have pointed out that during the period of initiation of brain potentials there are also indications, from the change in the behavior of the fetus, of the beginning of function in the higher parts of the nervous system. Kimel and

Kavaler (12) in an investigation, as yet incomplete, of the onset of peripheral response following cortical stimulation have demonstrated that all the peripheral responses elicited in a series of adults can be obtained in a fetus approximately 55 days old. Thus there is evidence that the axons of the cortical nerve cells reach their destination in less than 10 days after active sprouting is first observed. A measure of the rapidity of growth of these axons can be obtained from these observations. Those which end around the cells of origin of the motor nerves of the hind limbs grow a distance of 6 or 7 cm. in approximately 10 days.

We take the series of concomitant cytological, biochemical, and physiological changes which I have noted to signal the differentiation of the neuroblast into the neuron. Hydén (13) has described another important change which appears to fit this pattern. Using the ultraviolet microscope, he found that the nucleolus can be seen for the first time when the neuroblast is actively synthesizing protein and there is a simultaneous increase in cytoplasmic nucleoprotein. These findings, together with similar ones on damaged adult neurons in process of reconstitution, led to the highly stimulating view that the nucleolus and the nucleic acids of the cytoplasm are responsible for the [illegible]

a [illegible] to the problem of nerve regeneration. It is a tribute to the insight of Van Gehuchten that in 1899, as a result of studies of Nissl preparations by himself and his student, Van Biervliet (14), he inferred that the chromatolytic, regenerating nerve cell returns to the embryonic status. Recent studies of enzyme activities in regenerating neurons fit this thesis. Howe and Mellors (15) found that the activity of cytochrome oxidase is reduced in the chromatolytic cell; Bodian and Mellors (16), that the activity of acid phosphatase is increased; Howe and Flexner (17), that the activity of succinic dehydrogenase is decreased. These are the changes which would have been predicted from Van Gehuchten's generalization and from the findings on the immature nerve cell (1, 2, 7). Nor is this surprising, in view of the fact that both the regenerating and the immature nerve cell have a common function, the synthesis of neuroplasm.

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with an increase in the activity of cytochrome C, followed in a few days by an increase in the activity of succinic dehydrogenase. In the guinea pig, Belknap (6) has found the same relationship with respect to succinic dehydrogenase. J. B. Flexner, in her continuing study of the energy relationships during growth and differentiation, has found that the enzyme adenylpyrophosphatase, believed to make energy available to the cell from energy-rich adenosine triphosphate, has a low activity up to the forty-second day of gestation and then begins rapidly to increase (7). It is clear, consequently, that cytological changes which include the appearance of Nissl substance, growth of the nucleus to its final volume, and rapid sprouting of processes are accompanied by demonstrable changes in enzyme activities.

What can be said of the functional differentiation of the cortical nerve cells? This problem has been approached in three ways: by recording electrical potentials from the cortex, by observing peripheral response to electrical stimulation of the cortex, and by studying the behavior of the fetus.

In a study which is of great interest to us, Jasper, Bridgman, and Carmichael (8) found that they were first able to record electrical activity from the cortex of the fetal guinea pig at the forty-eighth day of gestation. Tyler, Gallant, and I (9) have confirmed these results, with the addition that we have recorded activity as early as the forty-sixth day, the end of the period which has been shown to be critical for cytological and biochemical differentiation. Efforts are now being made to determine whether these potentials are partly or wholly cortical in origin. In her biochemical studies, J. B. Flexner (10) made an observation which may give considerable insight into the events responsible for the onset of electrical activity. Up to a gestation age of 41 days, chloride and sodium are distributed in the same volume of cortex; both are apparently confined almost completely to the extra-cellular phase. At the forty-sixth day and thereafter, sodium is distributed in a considerably larger volume than chloride. It is tempting to ascribe this increase in permeability to sodium to the nerve cell and to suppose that the onset of cortical electrical activity is related to the appearance of a selective permeability to sodium, as has been hypothesized by Hodgkin and Katz (11) to account for the action potential or peripheral nerve.

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SOME ASPECTS OF NEURAL GROWTH, REGENERATION, AND FUNCTION

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IN THE nervous system, spatial and temporal considerations dominate as in no other system. For all tissues, including neural, the anatomical patterns and the chemical processes underlie both development and function. At present, development and function tend to be studied by separate groups—the embryologists and the physiologists; but this is as unsound as other dichotomies of being and becoming (1).

Essentially the same organismic influences operate in both, to modify pattern and process in time, whether the change is normally unidirectional, as in development, or normally reversible, as from rest to activity. And, with time and space so emphasized in the nervous system, it is not surprising that an especially effective confluence of seemingly disparate interests is occurring about this subject.

The assertion of the unique importance of space and time co-ordinates in the nervous system needs little justification. Molar organization is present in the circulatory, digestive, respiratory, and musculo-skeletal systems, but hardly above the mechanical level of pumps, ducts, and levers. Submolar organization of cells into secretory units exists in acini, liver lobules, kidney nephrons, and the like; but the further gathering of these tiny masses into large organs is simply trivial repetition. And micro and molecular organization is at a [illegible] in, probably, all cells. But micro and molar organization [illegible] The neuron has an extension [illegible] servient skeletal musc[illegible] its subentation, and establishes connections to a degr[illegible] sses orioses, qualitatively unique in the organism. Further, the neuron population is not an aggregate, like a mob, but an "org," like an army (2). The neurons are critically organized into particular nets and other interacting groups, so that a change in almost any part leads to definitive changes in many others.

Such spatial facts necessitate critical temporal relations, if nothing

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behind a temporary constriction. Young (11) likewise obtained direct histological evidence of material flow into axones; and Hydén and his colleagues (12, 13, 14) inferred, from changes in the ultraviolet absorption spectrum under conditions of activity, chromatolysis, etc., that nucleoprotein moves from soma into processes of the neuron. But the movement is actually more of a mystery than ever.

Endoneurial fluid moves some millimeters an hour. It is not pumped by the pulse pressure from the dorsal cavity, for movement occurs *in vitro*. Axon material moves a few millimeters a day, not far from the rate of growth of a regenerating axon. Is the entire axon, then, growing steadily during the normal life-span, like a rat's incisor, and, if so, what happens to it peripherally? Or does the axoplasm cylinder push distally in a fixed sheath? Or do particular substances migrate distad in the axoplasm? And here also arise questions of the driving force—the pulling-out of pseudopods along interfaces, osmotic turgor of the cell (which should be lower during active synthesis than at other times), an undefined growth process. These questions remain unanswered.

I can report, however, some further evidence of peripheral movement in axons. Soon after the war we started experiments with P^{32} [illegible]

[illegible] ...eeks, and the specific activity of four P fractions—acid-soluble, phospholipin, nucleoprotein, and phosphoprotein—was determined at appropriate intervals in brain, cord, and two or three segments of the sciatic nerve. It is not appropriate to report here the findings on rates of penetration of phosphate into these structures or rates of exchange of the phosphate of the organic compounds, although it bears mention that all are so slow that equilibrium is not attained in 2 or 3 weeks (15). We hoped to find, if spatial migration were rapid enough compared to chemical turnover, that labeled nucleoprotein, formed in the cord neurons, subsequently moved down the peripheral processes. In this we were disappointed; nucleoprotein P exchange occurred at comparable rates in cord and nerve, and no tide of high specific activity of this fraction moved along the nerve. Surprisingly, however, just such a phenomenon was encountered in the pho[illegible]

of the top seg[illegible]

high at first [illegible]

14:1; during th[illegible] during the first 10 days the ratio [illegible]

Despite wide s[illegible]

more. The timing of events, be they the movements of growth cones in morphogenesis or of impulses in behavior, must be exquisitely precise. Spatial patterns require temporal ones both for their formation and for their function—a logical expectation amply documented by the facts of neuroembryology and neurophysiology.

The unique problems of the nervous system, then, center about the interrelation of events separated in space and time and involve the interaction of distant regions, the communication of information, and the issuance of commands. The mechanisms of such distant interaction—between cell soma and process or between separate cells—thus assume paramount interest for us. I shall not consider here the interaction of neurons (3, 4, 5), whether as nets or as synchronized groups, but shall direct attention to the cell body–cell process relations.

When a nerve fiber is cut from its cell body, the amputated axis cylinder dies, and the soma passes through a more or less severe chromatolysis. How does a section at the knee make itself known to a soma in the cord and to a terminal in the foot? What is lacking for the separated fiber? Some transmitted influence or transported substance, normally supplied by the soma, must be necessary to the integrity of the fiber. We attempted some years ago (6, 7, 8) to distinguish between these categories, and all evidence favored the actual transport of substance. A cut nerve, kept active conducting impulses, degenerated sooner than one at rest, as if needed material were being exhausted more rapidly rather than as if some isolation dystrophy or atrophy of disuse were being avoided. The converse situation—of a nerve under pressure for months with conduction blocked—did not lead to fiber degeneration, again indicating the transport of material along an intact protoplasmic connection. Finally, the rapid passage of virus and toxin along axons and the observed "peristaltic" movements of axons, that might account for such passage, made chemical transport a reasonable mechanism. Since nerves are independently supplied with a circulation that can bring substrates and remove wastes and since the nucleus of the soma is its most distinctive organelle, it seemed a fair guess that an enzyme formed by or under the influence of the nucleus must continuously pass along the fiber to keep up its metabolism.

More recently, direct evidence of the peripheral movement of material of the axon has come from several sources. Weiss *et al.* (9) showed rapid transport of tracer ions by endoneurial fluid, and Weiss (10) also found a slower transport of axoplasmic contents, piled up

the frog spinal cord following section (22), we undertook the measurement of oxygen consumption of single spinal ganglia under various conditions (23). This study was interrupted by the war and has not been completed or fully published, but suggestive results were obtained.

A rat spinal ganglion weighs about 0.6 mg., and its respiration can be followed readily in a capillary respirometer (24, 25). Oxygen used per hour per normal ganglion is reasonably constant at 0.9 cu. mm. (This represents an average of 5×10^{-5} cu. mm. per neuron per hour.) Ganglia measured at 5–25 days after section of the proximal dorsal root showed no clear change in respiration rate (Fig. 1). After distal section of the dorsal root (which, in contrast to proximal section, does produce chromatolysis), a sharp rise in oxygen consumption resulted. This averaged 1.3 times normal over the first 10 days after section and seemed elevated even after a month (Fig. 2). The data are too few to be certain of quantities and time relations, but that a considerable increase in metabolism occurs after section of the distal cell process but not after section of the proximal one seems reasonably clear.

Others have followed changes in substance content and enzyme activity of the spinal gray after root or nerve section and have found increased phosphatase (19) and decreased nucleoprotein (13, 26), cytochrome oxidase (27), creatin phosphate (20), and cholinesterase (28).

In such studies, as to some extent in the ganglion studies, the combined mass of the neurons is diluted in the tissue sample by the [illegible]

[illegible] cell bodies of neurons are reported to occupy only 2.85 per cent of the total volume (29), the remainder being mostly glia. Even in lower forms, neuron somas occupy but 12 per cent of the total cortical volume, though perhaps a considerably larger fraction of the total protoplasm (L. Flexner, personal communication). Glia, according to our estimate from the filum terminale (23), has a respiration rate similar to that of neuronal structures. It is hazardous, therefore, to draw metabolic conclusions about neurons from measurements on such mixed central nervous system tissue samples. Conversely, the tendency to relegate glia to a casual structural role in the nervous system is surely myopic. I suspect these cells are important in nutrition—though the "sucker-foot" approach leaves much to be desired—and I know of no evidence that excludes them from a role

vancing tide being illusory was calculated at $P = 0.002$. The rate of advance of the high-activity phosphoprotein is 2.5 mm. a day, essentially that obtained by Weiss for movement of axon material.

We also tried the effect of malononitrile, reported by Hydén and Hartelius (14) greatly to accelerate nucleoprotein turnover. Even under their conditions and at time intervals of from 4 hours to 18 days, we were unable to find any change of absolute content or specific activity in the chemical fractions or of specific activity in any of the gross tissue samples tested. (Their measurements, of course, were on single somas.) Nerve section, on the contrary, does lead to marked changes in the separated nerve, lesser ones in the cord. Turnover is promptly increased, and, by the second day, the specific activity of nerve phospholipins and phosphoproteins is twice the normal, and that of nucleoproteins is tripled. Acid-soluble phosphate shows no change—a good control. Activities are still high at 2 weeks, by which time the actual content of phospholipin is halved and that of nucleoprotein is doubled. One may, of course, attribute the greater activity and amount of nucleoprotein to the multiplying Schwann cells, and the rapid loss of phospholipin to myelin catabolism; but why the necessary enzymes for these processes should become activated by the loss of something coming from the soma remains a problem. Perhaps clues exist in the activation of glycolysis and of proteolysis by the suppression of normal oxidations.

Turning, now, to the cell soma, it is hard to believe that changes in it, after fiber section, can be due simply to any deficit. Some signal must travel retrograde from the cut, and it is, of course, generally accepted that chromatolysis is more severe with a proximal, than with a distal, cut (16). Yet section of the central process of a spinal ganglion cell (17), perhaps of any dendrite (18), causes little or no chromatolysis, despite close injury. What cue reaches the cell body and how it initiates the cycle of chemical and morphologic changes (18, 19, 20, 21) that constitute chromatolysis and recovery remain mysteries. It is not even clear whether these changes are degenerative or reparative in character.

A long axis cylinder may contain a thousand times as much protoplasm as its cell body; and, during regeneration, axoplasm is formed [illegible] times its volume [illegible] during regeneration implies increased metabolism and enzyme activity, which must be established during the transition period of chromatolysis. On the basis of such reasoning and the report of an increased respiration of

in neural function, even in impulse conduction. It is reported, rather, that preganglionic stimulation leads to glial hypertrophy in autonomic ganglia (30). Dr. Tschirgi, who holds similar views, is, accordingly, initiating an intensive study of the glia from the physiological viewpoint.

Important activity of glia in pathological changes in the central nervous system, by contrast, is well known. This introduces the problem of the nonneuronal elements and regeneration. That neurilemmal bands and tubes offer important directive action in the outgrowth of regenerating fibers is now well established (10). Whether they supply only, or more than, a mechanical scaffolding is not certain, though the former seems probable, especially in view of the mysterious influence of the periphery (31). Further, the gross distribution of fiber bundles and connective-tissue septa in a nerve trunk are important in determining the success and degree of regeneration (32). *To what extent do the glial elements enter the picture of regeneration in the central nervous system?*

The standard teaching has been that cut fibers do not regenerate within the central nervous system; and surely such regeneration is at a different level of success than that in peripheral nerve. A somewhat circular argument has resulted: central regeneration cannot occur because Schwann cells, essential for regeneration, are absent; the proof that Schwann cells are essential is the failure of regeneration in the central nervous system, where they are absent. Actually, of course, oligodendroglia cells seem to have much the same anatomical relation to central fibers as do Schwann cells to peripheral fibers. Further, it is an indubitable experimental fact that cut fibers regenerate centrally as they do peripherally. The question thus becomes, "Why is this central regeneration so frequently abortive?" rather than "Why does it not occur?" The answer, I believe, is not in limited potentialities of the neurons but in an inimical environment.

There is no need to review here our own evidence (33) and that of others (but see Ref. 34, p. 186) for the successful regeneration, anatomical and functional, of the fully transected spinal cord of the rat and lower forms. These positive results, although often given verbal acceptance, have led to little further effort or clinical exploration. I am happy to learn from Dr. Windle of the recent positive results obtained in [illegible]

[illegible] on a tenfold larger series of animals.) Analysis is needed so as to convert a 10 per cent success into a 90 per cent one. Although

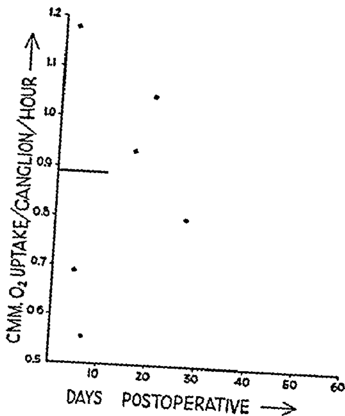


Fig 1 —Section of central root

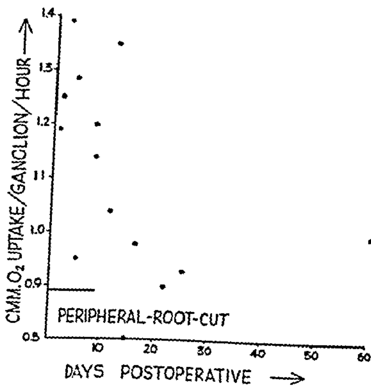


Fig. 2.—Section of peripheral root

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anoxia may play some role in the failures, as Dr. Hooker suggests, I am more inclined to blame the glia. It is hard to account otherwise for the poor regeneration of root fibers cut in the glial zone and their good regeneration when cut, just peripherally, in the neurilemmal zone (36). (Avulsed ventral roots do, however, regenerate [37].) An intense and early glial overgrowth occurs in the region of injury, and the sprouting nerve fibers probably become inextricably tangled in the scar, if, indeed, the glia does not damage neuron somas (30). Like the storied drunk, bumping time after time into the tree before his front door, they are "lost in an impenetrable forest." Devices, such as x-radiation, for hindering glial growth should favor central regeneration.

The few points touched in this discussion raise, rather than answer, problems, which is common enough, and emphasize the need of better methods that permit a finer correlation of chemistry with morphology; for, as indicated at the start, the metabolic and structural underlay of morphogenesis and of functioning are alike. Neural metabolism can increase some two- or threefold in passing from rest to activity, and a comparable change occurs during regeneration. Similar enzymes and intermediates undergo change on stimulation or injury, and the same problems of the control of enzyme action and substrate availability (38) arise in both cases. Indeed, growth and patterning, like hypertrophy and memory, are long-lasting consequences of function (39). One need only shift the time scale to pass from the physicochemical basis of nerve-impulse conduction to that of nerve-fiber growth.

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The single noteworthy exception is the published report of Sugar and Gerard, which states that the transected cord of adult albino rats exhibited regenerative phenomena, mainly fiber outgrowth, which re-established connections through which function was reasonably restored. The introduction of degenerating sciatic nerve between the cut ends of the cord facilitated the formation of the connecting fiber cables. The type of regeneration reported by Sugar and Gerard resembles that seen in older frog tadpoles, without the aid of "foreign" material. It is quite different from the observed results of fetal rat experiments. Were it not for a rather extensive personal experience with experiments on transection of the cord of fetal and adult rats, as well as of other forms, this report might appear less dubious. Nevertheless, it remains a challenge demanding further study.

The work with teleostean and amphibian forms presents a strong possibility that the varying capacity for regeneration in the central nervous system is closely related to the presence or absence of redox enzymes within the system. Apparently, this aspect of the problem has never been studied, but it unquestionably presents an important lead for future investigations for several reasons.

In addition, direct factual data are needed on the regenerative capacity of the central nervous system in reptiles and birds at various ages.

SPINAL CORD REGENERATION

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AMONG several incompletely solved problems of the nervous system is that of central nervous system regeneration. The starting point for all experimental work in this field is found in the studies of numerous investigators who have operated upon the early embyros of amphibian forms. In these, at the early stages, a skilful operator may alter the form of the nervous system in many ways and secure healing, often *per primam*, and complete restoration of form as well as of function. Relatively little has been done on the regenerative capacity of the brain, in so far as an organized attack upon the problem is concerned, most of the studies having been confined to the spinal cord.

With remarkably few exceptions, there seems to be general agreement on the following points: (1) in the teleosts, amphibians, and possibly birds, the spinal cord will frequently re-establish form and function after transection or other injury in early embryonic stages; (2) in certain teleosts, at least, the spinal cord retains much of its regenerative capacity well into the adult period; (3) in tailed amphibians, a similar retention of the capacity of the cord to regenerate is present for a varying time following metamorphosis; (4) in amphibians which are tail-less after metamorphosis, the spinal cord may regenerate at least until loss of the tail, there being relatively little evidence available on later stages; (5) in all these forms, regeneration becomes less complete, both morphologically and physiologically, with increase in age, and the number of individuals exhibiting regeneration, never 100 per cent, decreases markedly; (6) knowledge of the regenerative capacity in birds is slight and confused. In this region further factual information is needed

[illegible] very different [illegible] ith a single exception of note, all the results of experimental studies on infra-human forms and of a vast amount of clinical experience in man have indicated that the spinal cord lacks regenerative capacity except possibly for an extremely short period in early embryonic life.

Ependymal elements migrate by amoeboid movements and obstruct the lumen of the central canal, which assumes a spindle-shaped dilation However, when the regenerated spinal cord has grown for some length into the cartilaginous tube and already shows a certain degree of organization (stratification of white and gray matter), it starts to regress, presumably because of its lack of peripheral connections. As a result of this involution, the spinal regenerate, which had already acquired several layers, is reduced to the thin-walled ependymal tube described by previous investigators.

As mentioned before, the innervation of the regenerated tail is provided by the three last pairs of ganglia remaining *in situ*. Terni discovered a marked hypertrophy in the cells of these ganglia and ascribed it to their increased activity resulting from the expanded area of innervation. It was possible for me and Zannone to observe similar behavior in the motor cells of the ventral roots. A few days after the amputation, which involves the transection of the nerve fibers, the cell bodies start to show marked signs of hypertrophy, accompanied in some cases by chromatolysis. The cells will then retain their enlarged size, evidently in connection with their augmented functional load. A remarkable accumulation of tigroid substance can be demonstrated in these cells, indicating a high content of ribonucleic acid.

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SOME COMMENTS ON REGENERATION IN THE CENTRAL NERVOUS SYSTEM

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THIS is to report some observations on regeneration in the nervous system of urodeles and reptiles following amputation of the tail. In urodeles (*Triton*) transection of the tail is followed immediately by the formation of a blood clot at the wound surface. This clot soon becomes invaded by connective tissue, and at the same time the elements of the ependymal layer of the spinal cord undergo conspicuous changes. They assume amoeboid form and with active movements migrate toward the cut end of the spinal cord, where they close over the central canal. Subsequently, a phase of active proliferation sets in. The mitotic activity, restricted to the ependymal zone, starts at the very tip of the stump, where the canal shows a bulbous enlargement. As the ependymal canal elongates, some karyokinetic activity also appears along its walls, although the greatest number of mitoses is still found at the tip, which resembles an apical meristem.

At first, the newly grown nerve tube appears very irregular in shape and caliber; but when the regenerated tail has reached a certain degree of development, the walls thicken, and the whole structure becomes more regular. The spinal ganglia of the regenerated tail appear to derive from cells which migrate metamerically from the neural tube.

As for the Sauria (*Lacerta*), it has been known for some time that the spinal cord does not take part in the regeneration of the tail. The new tail differs from the original one also by the fact that its skeleton develops as a continuous cylinder of fibrous cartilage instead of as a metameric structure. Since no new ganglia are formed in the regenerated tail itself, its innervation comes from the last three pairs of spinal ganglia, which have remained intact.

It was possible for me and my co-workers, Marotta and Zannone, to show that in lizards the first phases of regeneration of the spinal cord are essentially the same as in urodeles but that during a later stage degenerative processes supervene. After the amputation a blood clot forms, which then becomes invaded by connective tissue.

tive to tactual stimuli, followed within a very short period by the ap- [illegible] supplied by the mandibular divi- [illegible] flexogenous.

The responses elicited by light tactual stimulation are not local, nor are they simple in type. They occur at a distance from the site of stimulation, usually on the contralateral side. Although the period during which they may be elicited is markedly shortened by interference with the placental circulation, the essential character of the reflex is not altered for an appreciable time interval.

With the progressive development of neuromuscular connections, the responses expand, chiefly caudally, to involve a larger portion of the trunk in a reflex of the same character as that first appearing. The integration of the expanded reflex, as of all subsequently appearing responses, precedes the appearance of the added areas involved and is an essential component of the normal processes of development within the nervous system. Integration is not secondary in the view of this group, nor can it be.

As the nervous system continues its growth, localized specific reflexes progressively appear. As a result, the essentially total character of the initial response pattern is modified. It is believed that these specific responses are not in any sense "simple" but that they are made possible by the increased number of new nerve fibers and the development of many new synaptic connections within the nervous system. The appearance of specific responses is accompanied by an expansion of the reflexogenous areas of the skin Ultimately, most of the surface of the fetus becomes sensitive to stimulation, and this expansion is accomplished in a generally distal and caudal direction.

Despite the difference of opinion, there is a considerable area of reasonable agreement between the two groups, largely relating to the period before the appearance of true or doubtful reflexes, as follows: (1) the musculature reaches a level of development at which it may be electrically or mechanically excited to contraction at a time which precedes the appearance of function in the neural mechanism; (2) the motor nerves precede the sensory in their capacity to transmit nervous impulses, and their electrical stimulation may cause muscular contractions not long after the muscle may be electrically or mechanically stimulated to contract; (3) the sensory and very possibly the intercalated elements of the reflex arc are the last to become functional; and (4) it is highly probable, if not certainly demonstrated, that proprioceptive reflexes antedate the exteroceptive in appearance.

NEURAL GROWTH AND THE DEVELOPMENT OF BEHAVIOR

DAVENPORT HOOKER

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THE sixth session of the conference clearly demonstrated the differences in interpretation from essentially identical observations that have created two opposing and, so far, irreconcilable points of view regarding the initiation of exteroceptive neuromuscular responses in mammals, especially in man. These divergences indicate all too clearly the need for further intensive investigation of the roles played by embryonic and tissue respiration in limiting or abolishing reflexes, of the nature and extent of the early-developing tract systems, and of the character of the stimuli which may evoke reflexes. Some of these studies must be physiological, some morphological in nature, and still others must be attacked by both approaches.

All investigators in this field have observed localized, sometimes apparently simple, but at other times quite complex, responses to stimulation of various kinds. One group interprets the simpler types of responses as the fundamental reflexes from which the more complicated behavior forms are built up by the addition of successively added simple reflexes, each of which is subsequently integrated with those which have previously appeared. Furthermore, this group is

connected by an intact placental circulation. They maintain that all responses elicited at whatever early age when the placental connections have been disturbed are abnormal, showing the effects of anoxemia and elicitable only from asphyxiated embryos and fetuses.

The other group of investigators believe that all early "simple" responses are not true reflexes at all but are caused by direct mechanical stimulation of the muscle tissue underlying the extremely delicate embryonic and early fetal integument. This group believes that the earliest true reflexes involve the entire functional neuromuscular mechanism then available in response to light stimulation of a tactual nature within a relatively circumscribed area in the facial region. Indeed, this group believes that the skin area supplied by the maxillary division of the trigeminal nerve is the first to become sensi-

anesthesia of varying depth, and the first ones of Barcroft and Barron were done under urethane. Investigators in Carmichael's laboratory and in mine, using the guinea pig and cat, avoided anesthesia during experiments by previously sectioning the spinal cord or decerebrating the pregnant subjects. Nevertheless, in my own and probably in other early studies, delay after incising the uterus usually allowed anoxemia to develop before observations were well begun.

One must evaluate critically much that has been written about behavioral development, bearing in mind the methods employed in the experiments. The use of great numbers of specimens and the averaging of data are not substitutes for judicious planning of experiments or critical physiological observations. The use of technics of decerebration, cord section, and local and spinal anesthesia, together with speed of observation and careful handling of the uterus, ultimately led to observation of the type of fetal behavior which I shall describe.

Significant species differences appear in the course of the development of somatic activity, but in early stages of all that I have had occasion to examine (rat, guinea pig, sheep, cat, and man) there appear to be remarkable similarities A certain amount of generalization would seem to be permissible.

The first somatic movements can be elicited by adequately stimulating embryos shortly before the time at which external morphologic characteristics of the species become recognizable, i.e., before the period of the fetus is entered upon. However, there is no evidence that embryos or young fetuses perform any movements spontaneously *in utero*. Not until approximately 14 weeks can intra-uterine movements be detected in the intact human being. The mother cannot feel them with certainty until about 17 weeks. How much earlier they normally begin I do not know.

When the abdomen of an experimental animal is opened and the gravid uterus exposed without handling, the fetuses within it appear singularly quiet. Other investigators have reported similar observation. In the sheep, an especially suitable animal for the purpose, Barron found by transillumination that so long as the uterus was unmolested the embryo was inactive. Manipulation during delivery led to activity. In the cat, changes in uterine tonus and impairment of oxygenation of the fetal blood led to activity in otherwise inactive fetuses.

Striated muscles develop before spontaneous movements can be observed and before reflex movements can be elicited. It is possible to induce movements of developing muscle masses of the shoulder

REFLEXES OF MAMMALIAN EMBRYOS AND FETUSES

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DEVELOPMENT of behavior is a broad subject, and I can touch only upon the aspects of it pertaining to the initiation of motor functions of mammalian embryos. Concerted efforts to determine how the nervous system of the embryo begins to function were begun approximately twenty years ago. They followed a series of reports of the genesis of nervous activity in amphibian embryos, notably investigations by Coghill, who accompanied his study of function with detailed histological studies of the developing nervous system.

When I became interested in this subject, about 1928, several other investigators had published reports on the activities of early mammalian fetuses. Some of these were scattered descriptions of one or two human specimens, but Minkowski in Switzerland and Bolaffio and Artom in Italy had assembled significant numbers of observations. In this country Swenson and Angulo were studying the motor activities of embryos of the white rat. These studies are interesting and present facts of importance, but there is not time to describe them.

All these early experiments, as well as those which Griffin and I performed and even some by more recent investigators, are subject to criticism because the experiments were conducted under unphysiological and abnormal conditions—indeed, usually on deeply asphyxiated and dying fetuses. At the time that the early studies were made, so little was known of intra-uterine physiology that observers were insufficiently concerned about their ability to control their experiments. Misconceptions of fundamental principles were commonly held, narcotic or anesthetic drugs which depress or abolish reflex activity were frequently employed, and effects of asphyxiation were seldom considered. Minkowski often used local anesthesia for his human patients, but the fetuses were removed from the uterus before they were studied, and there can be no doubt that asphyxia prevailed. The same criticism applies to Hooker's studies in man. The rat experiments of Swenson and Angulo were carried out under ether

employed. Except in the earliest phase of anoxemia, a fore-limb reflex usually cannot be obtained in small fetuses by light stimulation of a single point, although stronger stimulation of a greater surface may elicit one. Stretch and pressure are more effective than light touch. If the fore-limb reflexes are muscle-stretch reflexes, one should not expect to elicit them with light stimuli.

The early reflex movements of embryos differ from movements induced by direct stimulation of muscles in many ways. The fore-limb reflex of the youngest embryo can be obtained only once, as a rule, but the limb will move repeatedly when its muscles are stimulated electrically. The fore-limb reflex is obtainable for only a few moments while physiologic conditions are best. When more than one reflex of the fore limb is elicited from an embryo, the second movement cannot be obtained until a brief interval has elapsed after the first; the mechanism for the response appears to be momentarily fatigued. The reflex is always a movement of one type occurring in one direction, whereas the movement in response to direct stimulation of the muscle is readily molded by the location of the stimulus. Although asphyxiation quickly abolishes reflex movements of the fore limb, it usually changes the appearance of the head reflexes before they are abolished; for example, contralateral movements become homolateral. More will be said about this reversal later.

In cat embryos whose afferent neurons have grown out distally into the limb beyond the region in which muscle cells have differentiated, it is possible to stimulate these nerve fibers without stimulating the more proximal muscle masses. When this is done, but *only* during the time that normal physiological conditions prevail in the embryo, a reflex movement of the fore limb can occasionally be induced.

After reflexes are no longer obtainable, one can insert a fine electrode into the spinal cord, stimulate, and obtain movements of the fore limb of a cat fetus. A forward movement results from stimulating in the rostral part of the cervical enlargement, and a caudal movement of the limb results from stimulating the caudal part of the cervical enlargement. When a stimulus is applied in this manner to the thoracic or lumbar portion of the spinal cord [illegible] lows if the em[illegible] ed. Howev[illegible] adequately [illegible] surface over the cord in the lower thoracic regions induces movements of the fore limbs. This is interpreted as evidence of conduction

region by electrical stimulation of the muscles themselves. The embryonic muscles of this region receive fine nerve endings from the spinal nerves at this time, but the contractions induced in this way have certain characteristics that distinguish them from true reflex movements.

Early reflex movements, involving conduction through the nervous system, are elicitable about 1 day after it is possible to obtain muscle contractions by stimulating shoulder muscles directly. The earliest responses of this type are movements of the fore limb or movements of the head. In the cat embryo a fore-limb reflex is present in some embryos that show no head response. When head movement and fore-limb movement are both present in the same specimen, they are not co-ordinated, and one or the other can be elicited independently. As development proceeds, it is possible to obtain similar unco-ordinated movements in other regions.

The nature of the early fore-limb movements has been questioned. Some investigators have doubted that they are true reflexes and have suggested that they may follow conduction of impulses to the muscle over motor neurons but involve no sensory neurons and no reflex arcs. My experiments indicate that they are reflexes.

The movements can be elicited in a number of ways. They do not occur spontaneously but follow external stimuli, provided that the stimuli are adequate. When conditions as nearly normal as possible prevail in an experiment and the uterus is opened quickly to display the embryo within its amnion and still attached to the uterine wall by its placenta, tapping or lightly pressing upon the amnionic sac often induces a quick outward and backward twitch of the fore limb. A similar movement can be obtained by passing a sharp needle through the amnion and flipping the limb gently with it. Once in a while it is possible to obtain a similar response simply by touching the tip of the fore limb with a hair.

Another somatic movement that makes its appearance almost as early as the fore-limb reflex is an extension of the head backward or to one side (usually the contralateral) when the nose or mouth region is touched or stimulated electrically. The movement of the head in response to external stimulation is more resistant to changing physiologic conditions than is that of the fore limb. The fore-limb movement is more easily abolished by anoxemia than is the movement of the head. This may be one of the reasons why some investigators have failed to observe the movement of the fore limb while readily observing that of the head. Furthermore, adequate stimuli must be

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neuron reflex arcs are formed. Thus both two- and three-neuron arcs are developed coincident with the appearance of the first fore-limb reflexes.

A re-examination of the early stages in the development of movements in the unanesthetized cat under good physiological conditions has confirmed my former views. The earliest somatic activities of which the cat embryo is capable make their appearance about 23 days after insemination. They are the simple reflex responses to stimulation of the fore limbs or head. Additional simple responses, appearing during the following days of gestation, are elicitable independently of one another, even after progressive integration of older activities has been accomplished.

Further consideration was given to the relation of asphyxiation to fetal reflexes. By deliberately altering the quantity of oxygen or carbon dioxide breathed by the mother and by inducing asphyxiation in the fetuses, several pertinent observations were made. Cat fetuses become more irritable to light tactile or pressure stimuli during the early stages of impaired respiratory conditions. It seems that anoxemia at first facilitates reflexes in embryos which normally were quiescent, but, later on, it depresses individual movements. Some, especially those involving appendicular muscles, become lost entirely. As irritability decreases and stronger stimuli are required, spontaneous movements begin.

During asphyxial conditions, stimulation of a fetus results in mass movements. The more profound the asphyxia, the more tonic and sustained are these mass movements. Asphyxiation ultimately leads to a breakdown of the mass response and to depression of all activities.

Rhythmicity of certain movements, especially of the muscles to be employed later in respiration, appears in cat fetuses about 30 days old under conditions of anoxemia. The more prolonged the anoxemia, the more muscles come into the respiratory-like rhythms of movement until deep gasps alone ensue.

Conditions of fetal respiratory metabolism are easily disturbed, and individual reflex movements are likely to be abolished in young fetuses before adequate stimuli can be brought into play to elicit the reflexes. A spontaneous mass movement, commonly referred to as "totally integrated behavior," is often the only form of activity re- [illegible] ts are [illegible] results have been obtained in the human fetus. Fitzgerald

up the cord and synaptic transmission at a brachial segment in the cord, in other words, a reflex in which no receptor ending is involved.

Considerable anatomical evidence favors the reflex nature of limb movements of embryos and young fetuses. Embryos of several species of animals have been stained by the Ranson pyridine-silver technic, which is especially good for demonstrating young neurons. All nervous elements essential for reflex action are present in the spinal cord shortly before the first reflex movements can be elicited by stimulation. Afferent nerve fibers run in the peripheral nerves to the tissues immediately beneath the epithelium of the fore limb and to deeper structures. The central branches of these neurons constitute the dorsal roots and dorsal funiculi of the spinal cord. Motor fibers arise from well-developed ventral horn cells in the spinal cord and pass through the ventral roots to the muscle masses of the fore limb and trunk. These nerve fibers can be seen ending in simple terminations upon the young muscle fibers. Within the spinal cord there are well-developed *commissural and associational neurons* whose cell bodies lie in the dorsal part of the gray matter. The commissural fibers build the opposite ventral funiculus and come into close relation with motor neurons which supply the trunk muscles. Associational fibers course into the lateral funiculus of the same side and are closely related positionally to the dendrons of the motor neurons supplying the fore limb.

Before fore-limb movements can be elicited reflexly, the afferent neurons of the dorsal funiculus and the associational interneurons in the region adjacent to the dorsal funiculus lack connections adequate to complete reflex arcs. A few afferent dorsal root collaterals are present, but in no species examined (cat, rat, sheep, and man) do these nerve fibers extend very far into the gray matter. Reflex arcs are completed by further ingrowth of these fibers. A correlation can be drawn between the increase in number and length of collaterals and the occurrence of the first reflexes. Some of the collaterals have grown as far as the motor nerve cells and complete two-neuron reflex arcs.

The anatomical mechanism of the fore-limb reflexes consists of homolateral, unisegmental arcs. The first development occurs in the brachial segments of the spinal cord. Collaterals come off dorsal root fibers at this level of entrance into the cord. These synapse with the [illegible] the more medial commissural, [illegible] nd their axons to motor nerve cells supplying the fore limb of the same side. In this way three-

ment first appeared at 25 days, changing to a contralateral movement at 27 days. The reversals occurring earlier under the influence of asphyxia anticipated, as it were, the changes about to take place normally under better physiological conditions. Although asphyxia enhanced the irritability of the neurons at first, it ultimately abolished all reflex activity and exerted some selective action in doing so. The first reflexes to appear ontogenetically were not necessarily the last to disappear during asphyxia.

Observation of reflexes and spontaneous activities throughout development permit a limited amount of generalization. The mammalian fetus is essentially a spinal or bulbospinal animal in early stages. As gestation proceeds, higher centers in the brain reach a functional state. Even at the time of birth, the cerebral cortex is immature.

SUMMARY

Structural development permits somatic motor function to begin in the mammalian embryo shortly before the time at which the specimen takes on the external morphological characteristics of the species. The time at which movements begin spontaneously within the uterus is unknown, but it is probably days or even weeks after the first simple movement can be elicited by stimulation.

The first movement that can be obtained in an embryo is related to some maturation of its skeletal muscle fibers. Movements resulting from the stimulation of growing muscle masses are possible about 1 day before movements that require the presence of functional reflex arcs.

The first reflexes are of at least two types. Limb movements occur in response to stretching the tissues or putting pressure upon them. Head movements result from superficial light tactile stimulation in the face region. The limb reflex is a local homolateral movement, whereas the head reflex may be a contralateral movement. The difference between these two types of reflex is clearly explained by structural differences in the parts of the nervous system involved. A close correlation has been demonstrated between the time of appearance of the reflexes and the time of development and completion of reflex arcs in the central nervous system. These reflex arcs are made up of two- and three-neuron chains.

Impairment of respiratory conditions in the embryo has a profound effect upon the early reflexes. In the first moments of a declining supply of oxygen to the embryo, elicitation of reflexes is enhanced. As asphyxia sets in, reflexes are abolished or change their

and I have elicited movements at the operating table in nonanesthetized, nonnarcotized fetuses of about 8 weeks' gestation. While the fetus is receiving oxygenated blood from the intact placenta, its neuromuscular mechanism is excitable, and individual movements can be obtained by tapping upon the amniotic sac. During progressive asphyxiation these reactions cease, but responses of the trunk musculature to stimulation of the nose and mouth region are elicitable for several minutes after detaching the placenta from the uterus. At this time strong stimuli induce mass movements involving neck, trunk, arms, and legs.

One of the earliest reports of movements in human embryos of this age was that of Strassmann, who observed the activity of a specimen with placental circulation intact and apparently functioning. He saw movements of the arms and legs. Movements in only four other human embryos smaller than 30-mm. crown-rump length have been observed at the time of operation by others; asphyxia prevailed in all. Most older human fetuses were asphyxiated during the period of observation, and the mass movement was commonly seen. Only when physiological conditions are good, when anesthesia and asphyxia are avoided, can all the reactions of which the human fetus is capable be observed and correctly interpreted.

Another study of the relation of anoxia to early reflex movements of cat embryos has been made in my laboratory. Movements of the head in response to stimulation of several points upon the face were studied in nonanesthetized decerebrated cats. At first the circulation of the fetal blood to the intact placenta was maintained while responses to stimulation of the fetus were being observed. Later, the umbilical cord was clamped to induce asphyxia in the fetus.

The fetuses were relatively unresponsive when first delivered, but anoxemia, setting in upon opening the uterus, led to increased irritability in a few seconds or minutes. In the early stages of development, reflexes appeared after a short period of impaired oxygenation, although they had been absent a few seconds earlier. They were more readily elicited by stimulating a point and activating only a few receptors.

During the course of development and before the umbilical cord was clamped, the reflexes resulting from stimulation of receptors in the head region changed once or twice. For example, the pre-asphyxial response to stimulating an area above the eye was a homolateral head movement at 26 days of gestation, changing to a contralateral movement at 36 days. During asphyxiation the homolateral move-

ment first appeared at 25 days, changing to a contralateral movement at 27 days. The reversals occurring earlier under the influence of asphyxia anticipated, as it were, the changes about to take place normally under better physiological conditions. Although asphyxia enhanced the irritability of the neurons at first, it ultimately abolished all reflex activity and exerted some selective action in doing so. The first reflexes to appear ontogenetically were not necessarily the last to disappear during asphyxia.

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GENETIC NEUROLOGY AND THE BEHAVIOR PROBLEM

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ONE of the basic problems of genetic neurology is a description of the events and mechanisms involved in the development of the integrative capacity through which the nervous system functions to maintain the integrity of the individual by regulating (1) the chemical structure of the internal environment and (2) the intimacy of association between the sustaining (O_2 and energy sources) and destructive chemical and mechanical elements of the external environment. Of the manner in which the nervous system develops ontogenetically the capacity to regulate the structure of the internal environment very little is known at present, except for isolated studies on mammalian embryos and fetuses with regard to the genesis and maturation of the "respiratory centers," the vascular reflexes, and temperature regulation. Our more extensive knowledge of the development of the mechanisms regulating the association of the individual with the elements of the external environment—the development of overt behavior—is derived primarily from the basic studies of the late G. E. Coghill on the salamander *Amblystoma* and secondarily from studies during the last twenty-five years inspired by Coghill on other forms, including fishes, amphibians, reptiles, birds, and mammals.

As so frequently happens in a newly developed field of scientific investigation, the results of the pioneer studies gave rise to two opposing generalizations or descriptions of the manner in which the integrative capacity of the nervous system developed. One generalization was advanced by Coghill on the basis of his studies on *Amblystoma* and was supported by the work of his disciples on other forms, to the effect that "behavior develops from the beginning through the progressive expansion of a perfectly integrated total pattern and the individuation within it of partial patterns which acquire varying degrees of discreteness"; the second generalization, formulated by Windle and his co-workers on the basis of th...

character. A homolateral head movement may become a contralateral movement or vice versa. Rhythmicity of movement is another phenomenon occurring under duress. During asphyxiation all motility eventually ceases, but for some time before this occurs a mass discharge of nearly *all* motor units capable of functioning is easy to induce or appears spontaneously. This mass activity during asphyxia is what investigators see when they examine fetuses that have been removed from the uterus. It is the activity that has led some to the belief that behavior in man has its genesis in a "total pattern" like that of the larval amphibian studied so extensively by Coghill.

the axial musculature derived from the somite and supplied by the dorsal branches of the spinal nerves. The question at issue might be restated: "Does the neural control of the appendicular musculature develop in association with, or independently of, the axial system?"

The motor system of the spinal cord in all vertebrate forms thus far studied appears to differentiate prior to, and for some time independently of, the central processes of the sensory ganglia and the conducting elements of the alar plate. The differentiation of the motor neurons appears to progress temporarily in a craniocaudal direction without any evidence of segmentation; those segmental features of the cord that do develop—segmental ganglia, sensory and motor roots—are imposed upon the neural structures by the adjacent somites and their derivatives, the sclerotomes. The number of cells in the basal plate at any particular level of the spinal cord appears to be determined by the relative position of the region considered in relation to the long axis of the cord and independent of the peripheral field.

Further, though precise comparisons are not available, there appears to be a tendency for the limbs to appear earlier in some forms, relative to the development of the central nervous system, than in others, for example, the mammalian limb appears earlier than that of the urodele, viewed with respect to the differentiation of the nervous system. Whatever the stage of its appearance, the limb appears to determine the proportion of the cells in the basal plate of the associated regions of the spinal cord that differentiate into motor neuroblasts, and these cells that develop into motor neurons do so within the matrix of the existing neuromotor elements If the appearance of the limb is late, relative to the development of the spinal cord, as in the urodele amphibians, the neurons that differentiate in response to the growth of the limb must do so in a region in which motor neurons supplying axial musculature are already functional and active; whereas in the case of a placental mammal, such as the sheep, the limb develops very early, relative to the cord, and the organization and primary differentiation of the motor neurons to the limb appear to parallel that of the motor neurons to the axial musculature. Some neurons in both groups would appear to become functional at approximately the same stage in development. In intermediate forms the differentiation of the neuronal system to the axial musculature might be expected to precede that to the appendicular by an interval related to their places in the phylogenetic scale—the chick appears to represent such an intermediate stage.

combined to form more and more complex reactions until the final behavior patterns are established. The value of these two generalizations for the development of interest in and research on the development of the functional capacity of the nervous system cannot be overestimated, for science has always been advanced by wholesome controversy, but in some respects the limits of their usefulness would appear to be fast approaching, for they have served to focus attention upon specific details rather than to further broader interest in the general problem. After the review at the round-table discussions in Chicago last March of the data accumulated in support of the two opposing views, those interested in the field appeared to be faced with the following alternatives: (1) that Coghill's generalization applies to *Amblystoma* and other vertebrates up to and including the pro- and metatherian mammals but not to placental mammals or (2) that the basic pattern for development is the same in all vertebrates, though modified in detail by the evolutionary contraction, in higher forms, of the larval state—so prominent in *Amblystoma*—and the acceleration in the appearance of the limbs in the higher tetrapods relative to other body parts. Adoption of the first alternative requires the acceptance of the view that the principles upon which the higher nervous system is developed differ fundamentally from those in lower forms—a view not easily reconciled with the great body of knowledge illustrating the operation of common principles in the ontogeny of the nervous system of birds, marsupials, and placental mammals. Following the second alternative, the two theories may have arisen as the result of emphasis upon certain aspects of difference between the development of behavior patterns of lower and higher forms—differences that are primarily a function or consequence of some other aspect of development. The following considerations resulted from an exploration of that possibility; their unsatisfactory nature is freely acknowledged, but they are recorded in the hope that they may encourage others to similar and more successful attempts to resolve the controversy regarding the development of the integrative capacity of the nervous system.

Though the issue has never been stated in precisely these terms, one of the basic differences between the "total-pattern" exponents and the "isolated-reflex" group would appear to center about the genesis of the activity in the appendicular musculature, for all those who have described the early activity of embryos—whatever their position in the phylogenetic scale—appear to accept the principle of the total pattern with regard to the development of the activity of

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The temporal relationships of the differentiation of these two systems of motor neurons, axial and appendicular, would appear to have an important role in the determination of the character of the earliest movements of the embryo. Paul Weiss in a series of brilliantly conceived and executed experiments has shown quite conclusively that a muscle exercises an effect upon the associated motor neurons that increases or determines their central selectivity. Separated from their associated muscles by section of their axons, the motor neurons lose their selectivity and give rise to impulse discharges in circumstances in which they would not previously, when their axons were distributed to muscles, have been activated. If they are permitted to regenerate their axons into a muscle other than the one previously supplied, the central selectivity gradually returns, but it is of a character determined by the new muscle. In the early stages of the regeneration process, the firing of the neurons and the activity of the associated muscle is of an indifferent or gradual character, unrelated to the role of the muscle in development of the co-ordinated activity of the appendage. As the regeneration processes proceed, the responses of the muscle, at first general and diffuse, are gradually restricted because the central cells are, as Weiss expresses it, "modulated" by the muscle until the discharges of the motor neurons are selective, i.e., serve to integrate the activity of the associated muscle within the unit as a whole. This same process of "modulation" of motor neurons by muscles takes place in normal development, though the consequences of the process, because it appears to develop more slowly, the older the animal, are more readily demonstrated by neuronal regeneration.

In the light of these important facts it is clear that, in those circumstances in which the neurons of the axial musculature differentiate and are functionally active as the appendicular system develops, the motor neurons of the latter system might be expected to be activated before the modulation process was completed; as a consequence, the first movements of the limb would be associated with those of the axial musculature, and the association of activity would persist until the modulation of the appendicular motor neurons was complete. If, however, the appendicular and axial systems developed and differentiated at about the same time and at the same rate, the activation of the axial system would not be expected to be *invariably* associated with the appendicular system, or the activity of the appendicular system with the axial. Owing to the selectivity of the neurons in each system, depending upon the characteristics of the

central excitatory conditions, the axial and appendicular systems might act together or independently.

Thus the initial association between the movements of the trunk and the limb during the early development of *Amblystoma*, followed by the increasing dissociation, and the shorter period of association, together with the greater degree of dissociation between the axial and appendicular systems described in some mammals, might have a common basis. If these differences in the degree of associated activity between two systems of musculature can be so reconciled, clearly no new principle has been introduced with advancing phylogeny, so far as this aspect of the development of the integrative capacity of the nervous system is concerned; behavioral differences in development between species would appear to be the result of variations in the temporal sequence of the operation of mechanisms rather than to differences in mechanism.

This question of principle versus detail in development is of further importance with regard to the significance of the earliest limb movements in placental mammals. Opponents of the total-pattern and individuation theory of Coghill have pointed out, as stated earlier, that in the fetuses of placental mammals under appropriate circumstances the musculature of the limbs can be activated reflexly via their intrinsic afferents at the same stage—or just prior to it—in development that the limb muscles can be activated reflexly via afferents to the head. (The evidence in support of this contention, though extensive, is all of much the same character and far from decisive; but it is equally true that the evidence to the contrary is at the moment equally unsatisfactory.) On this observation the opponents of the Coghillian view have based their suggestion that the integrative capacity of the nervous system arises through the amalgamation of reflexes.

Assuming for the moment that the evidence for the existence of the intrinsic reflex so early is acceptable, the placental mammal would appear to be at the upper end of a phylogenetic sequence, the toadfish at the lower, with the urodele amphibian and the chick placed ... in that order. According to Cog... in the toadfish as in *Amblystoma*, ... xes out of the total pattern occurs with the difference that the period is much longer between the total-pattern fin reaction and fin reflex than in *Amblystoma* (1933). The interval is very much shorter in the chick, and it is debatable whether or not one exists in placental mammals.

The differentiation of the intrinsic afferents to the limb and the arrival of their central processes within the central gray of the spinal cord to complete the local reflex mechanism appear to be geared and timed by the structures that make up the limb. The more precocious the limb, the earlier the local reflex is established relative to the differentiation of the longitudinal or descending systems in the cord that are activated via the afferents to the head. Granted the evidence of the early development of the intrinsic reflex mechanisms of the limbs, is the difference between the expansion in the pattern of reactivity in the higher and lower forms one of temporal sequence or one of the nature of mechanisms?

The arrival of the intrinsic limb afferents within the cord to complete the local reflex arc not only provides for the activation of the appendicular musculature but also determines the degree to which its activity is isolated or segregated from all others, i.e., the degree to which the response is localized is a function of the inactivity of the other somatic muscles that make up the system. The de-afferented limb of a dog, for example, takes a greater part proportionally in the motor activity of the animal than in the normally innervated limb. Excitation, having arisen in a particular region of the cord, irradiates in an unlimited fashion, and the local limb efferents, in the absence of their intrinsic afferents, are activated by the spreading excitation. In the adult animal the intrinsic limb afferents limit or restrict the activity of the associated appendicular musculature. Similarly, in ontogeny, the arrival of afferents within the neuronal field of the limb efferents is associated—in the sheep, for example—with the dissociation of the limb from activity patterns of the trunk initiated through stimulation of the maxillary division of the trigeminal.

Clearly, inferences with regard to the principles of the development of the integrative capacity of the nervous system based upon observations of the activity of the embryo or fetus must be carefully drawn and after due consideration of the specialization of the animal type studied. In those forms with a short gestation period—the rat, for example—the intra-uterine period of reactivity is limited to 7 days, whereas in the sheep it is extended over 100. Discounting the differences in the degree of maturity at birth, the nervous system of the sheep appears to arrive at a stage of development equivalent to the rat at birth 30–35 days after it first becomes reactive. Under the circumstances the principles of development are more likely to be illustrated by the similarities in their activities than by the differences.

A further limitation to the value of reaction patterns as indexes of the development of the integrative capacity of the nervous system arises from the demonstration by Harrison and by Detwiler that neurons can differentiate in environments in which they cannot interact as units in a conducting system. This fact must be borne in mind whenever a comparison is made between the activities of forms developing in different environments.

The activity of the *Amblystoma* larva can be tested in the fluid medium in which it is normally developing, and this medium is one in which it lives when fully developed; there is therefore no reason to suppose that the maturing neurons are in contact with an internal environment other than that in which they will eventually interact as parts of a conducting system. Their interaction at any stage may, with a considerable degree of confidence, be taken as an expression of their full functional capacities. This same statement may be extended with full confidence to the activities of the "pouch young" opossum and with caution to the reptile and bird on exposure by opening of the shell. But the studies of the late Sir Joseph Barcroft and his collaborators have made it quite clear that the environment —the circulating fetal blood—in which the nervous system of the embryo or fetus of the eutherian mammal develops is quite different from the composition of the blood after birth.

In the fetal sheep, for example, aside from the last 10 days or so of gestation, the blood in the carotid artery is about 70 per cent saturated with oxygen and that in the confluence of sinuses about 50 per cent. Related to the dissociation curve and expressed in terms of pressure, the oxygen pressure in the carotid is but 33 mm. Hg, the pressure in the sinuses 30 mm. Accordingly, the mean oxygen pressure in the capillaries of the brain is about 25–26 mm. Hg (mean capillary pressure is estimated as follows: the difference in the pressure of oxygen in the artery and vein is divided by 3, and the figure so obtained is added to the pressure in the vein). During the last 10 days of gestation, by the same calculation, the oxygen pressure in the capillaries is 20–22 mm Hg.

Further, Miss I. M Young, of St. Thomas Hospital Medical School in London, who has determined the percentage saturation with oxygen of the blood from the carotids and fontanelles of fetal rabbits (1949), finds values that are of much the same order as for the sheep, with the trend in the fontanelles just a bit lower. Unfortunately, there are no dissociation curves of fetal and maternal rabbit blood available at present from which to estimate the pressures, but

they would appear to be of the same order as, if not actually lower than, those in the sheep fetus.

According to Mossman, the rabbit has a hemo-endothelial placenta—only one layer of tissue separating the fetal and maternal bloods; the sheep has a five-layered syndesmochorial placenta. Accordingly, the oxygen pressure found in the capillaries of the brain of the fetal sheep is not a function of the thickness of the placenta but is, in all probability, fairly characteristic of all fetuses of placental mammals. Moreover, these pressures, which would appear to be fairly representative of the whole of the fetal central nervous system, for the blood from the arch of the aorta via the carotids and vertebrals supplies almost the entire fetal neuraxis, are in marked contrast to those found in the adult. The oxygen tension in the adult carotid is about 85–90 mm. Hg, the pressure in the sinuses 55–60; accordingly, the pressure in the capillaries is about 65–66, more than double that in the capillaries of the fetal brain. In short, [illegible] fetus of the [illegible] ment, to fun[illegible] The amphibian nervous system, [illegible] other hand, differentiates in the internal environment in which it functions Simple inspection of the activity of the mature fetus exposed by Caesarean section before and after the establishment of ventilation of the lungs and the cessation of the placental circulation is sufficient to convince the observer that some of the conducting elements of the nervous system can develop in an environment in which they cannot function as units in a conducting system. *In utero*, with the placenta serving as the organ of gaseous exchange, the "near-term fetus" lies inactive except for an occasional sluggish movement; delivered from its membranes and using its lungs to oxygenate its blood, the newborn animal may, depending upon its state of maturity at birth, be able to stand, walk, and feed within a few minutes after being brought from the uterus, and its ability to do so—in the lamb, at least—[illegible] of the [illegible] ity of [illegible] the rise in the oxygen pressure in [illegible] reaching the brain. This change in behavior on delivery is clearly not due to any sudden advance in the organization of the nervous system but to a change [illegible] about the [illegible] units.

In co[illegible] it is difficult to accept [illegible] [illegible]

accordance with principles that are applicable throughout the entire phylogenetic scale; that view may simply be partly the result of an undue emphasis upon minor differences in expression at the two ends of the scale. In the physical sciences some laws or generalizations—Boyle's is an example—are not applicable over the entire range of circumstances which they purport to describe; yet the principles they enunciate are universally recognized. Coghill's generalization with regard to the nervous system may be a parallel in biology.

NEURONAL SPECIFICITY

R. W. SPERRY

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IN HIS Leipzig lectures of 1898, on the specific energies of the nervous system, Hering (1913) proposed that different classes of sensory and central neurons and the excitations which they transmit must differ from one another in quality. His proposal was based on the apparent impossibility of accounting for qualitative differences in sensation with only homogeneous impulses routed over different pathways. The central course taken by afferent impulses he conceived to depend upon the quality of the impulses as well as upon the anatomical pathways available. Much the same idea was expressed by Head (1920) and by other early neurologists on essentially similar grounds.

Qualitative specification of neurons has been inferred on an entirely different basis in early investigations of growing nerve fibers. The selective manner in which various fiber types manage to acquire their proper terminals in normal development and under certain conditions of regeneration led Langley (1898), Cajal (1928), Tello (1915), Harrison (1935), and others to assume that the different classes of neurons differ in their chemical makeup.

The subsequent failure, however, of electronic methods to reveal any important qualitative differences in the impulses conducted over different sensory pathways resulted in a general decline of interest in the concept of neuronal specificity and specific nerve energies. This trend was furthered by the demonstration that in many cases the apparent selectivity of nerve-fiber growth and termination could be explained in terms of stereotropic and other mechanical factors (Weiss and Taylor, 1944), without postulating chemotropic specificity.

Important new evidence of neuronal specificity was discovered by Weiss (1922–41), in a series of experiments dealing with the homologous function of supernumerary limbs in amphibians. These results greatly extended the earlier information and demanded reconsideration of the entire question. Motor fibers of the limb musculature were

demonstrated to differ qualitatively among themselves according to the particular limb muscle innervated. Similar specificity was found to be present also in the proprioceptive innervation of the limb muscles. Not only was the existence of neuronal specificity proved in these experiments, but it was shown, furthermore, to be much more extensive and refined than had previously been thought. What is more, Weiss was able to show that this myotypic specification of the limb nerves is brought about as a result of the peripheral contacts which the outgrowing fibers form with the limb musculature. Each muscle, it was concluded, possesses a chemical specificity of its own and imposes a corresponding specificity upon both its sensory and its motor nerve fibers. A similar capacity on the part of the cornea to specify its exteroceptive innervation was also demonstrated (Weiss, 1942; Kollros, 1943).

In addition, these experiments disclosed that the functional selectivity between central nervous system and peripheral end-organs is determined by the qualitative specification of the peripheral neurons. *After* various developmental alterations, the timing of the central discharge of the motoneurons regularly became adjusted to suit the particular muscles with which the fibers connected. When the peripheral connections were changed, the central firing shifted accordingly. With regard to the sensory nerves, likewise, the responses evoked by their stimulation were shown to be correlated consistently with the [illegible]

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fishes (Sperry, 1948*b*, 1949); and study of the function of supernumerary fingers in a human patient (Weiss and Ruch, 1936) has indicated the existence of similar relationships in man. Although it is to be expected that many of the details of neuronal specification and its role in neurogenesis as worked out on the amphibians will be found to vary in the higher vertebrates, the basic principles probably will not be subject to any radical modification.

Further evidence of neuronal differentiation can be found in the selective action on the nervous system of various viruses, bacteria, and other parasites; degenerative diseases; histological stains; hormones, drugs, toxins, and other chemicals. Variations in numerous other physiological and morphological properties are also indicative of qualitative specificity. In general, the type of nerve specificity demonstrated by phenomena of this kind is of a less refined order than that demonstrated by the methods involving phenomena of development and regeneration.

The evidence, on the whole, is now sufficiently extensive to indicate the presence of refined qualitative specificity throughout the entire nervous system. The sensory fibers have been found to be approximately as heterogeneous in character as are the elementary qualities of sensation which they mediate. Where local sign properties are involved, as in vision, pain, and touch, further qualitative differentiation is correlated with the topographic arrangement of the sensory endings. As mentioned before, the motor fibers differ according to the particular muscles which they supply (or major fractions thereof in the case of muscles with more than a single origin). In the autonomic system, also, there is good reason to believe (see Langley, 1898) that the pre- and postganglionic fibers are as diverse in their constitution as in their function. Within the centers likewise, chemical specification has been found to parallel closely the functional differentiation.

Cell differentiation in the nervous system alone is as multifarious as that of all the other tissues of the body taken together. In addition to the diversity of the primary motor and sensory neurons, which approaches that of the tissues they innervate, there exist multiple orders of extra specificity in the complex of association neurons within the centers.

It follows that the end-organ tissues must also possess a refined chemical differentiation far beyond what is visibly manifest. Not only is each muscle [illegible]

tendons, fas[illegible]

level motor systems are likewise governed by the constitutional specificity of the intra-central neurons.

The fibers of nerve VIII supplying the various vestibular endings of the inner ear (i.e., the cristae of the three semicircular canals; the maculae of utriculus, sacculus, etc.) are also specific in character according to the particular end-organs with which the fibers connect in the labyrinth (Sperry, 1945*b*). The central reflex relations formed by these fibers in the vestibular nuclei has been found to be regulated by this specificity in such a way that the central associations always match the peripheral terminals. Specificity is also indicated among the neurons of the vestibular nuclei on which the fibers terminate.

In the genesis of cutaneous local sign it is necessary for the cutaneous fibers to form functional relations in the centers that are precisely adjusted to the particular areas in which the fibers terminate in the skin. It has been shown that the cutaneous fibers are subject to a refined local specificity (Sperry and Miner, 1949). Experiments in progress (Miner and Sperry, 1950; Miner, 1950) indicate that the entire integumentum undergoes a refined fieldlike differentiation in development and that the local specificity of the cutaneous fibers is induced by the particular kind of skin in which the sensory fibers happen to terminate. When thoracic nerves are forced to terminate in the digital skin of a transplanted limb, they form reflex connections appropriate for limb digits rather than for thorax.

Additional experiments on the motor innervation of the extrinsic eye muscles have revealed a myotypic specificity similar to that reported by Weiss for the limb nerves (Sperry, 1947). The motor ocular neurons of IV and VI, however, become specified by self-differentiation, apparently, before the fibers reach their respective premuscle masses. The oculomotor neurons of III, on the other hand, seem to depend for their final specification upon contact with their respective muscles. Unlike the limb nerves, though, they lose the capacity to be respecified by foreign muscles at a very early stage of development. This difference between limb and ocular nerves suggests the possibility that neural specificity in the course of vertebrate evolution may have undergone an increasing degree of central self-differentiation and early determination.

Studies on neuronal specificity have been carried out thus far almost exclusively on the amphibians because of their recognized advantages for embryological experimentation plus the regenerative capacity of their central nervous system. However, results of the same kind have recently been obtained in the visual system of teleost

gray. The final specificity of a given neuron in many instances probably represents a complex product of the two processes.

The attainment of differentiation through terminal contacts with tissues far distant from the cell bodies releases the nervous system in some instances from certain of the limitations of differentiation to which other tissues are subject. In the ventral columns of the cord, for example, where there emerge in the limb segments as many different types of neurons as there are muscles in the limbs, it becomes possible for motor cells of quite diverse specificity to lie adjacent to and erratically intermingled with one another because their specification is acquired via axonal contacts with the musculature.

Thus far the evidence indicates that the pattern of specificity on either side of the sagittal mid-plane is a mirror image of that on the other. The neurons of the biceps brachialis, of the dorsal quadrant of the retina, or of the V sensory nucleus, for example, are apparently alike on right and left sides. If this holds throughout, it means that any given nerve cell could maintain only symmetrical relations on each side of the mid-line. Wherever asymmetrical excitation is initiated from unilateral stimulation, separate sets of neurons must be present to carry the impulses across the mid-line. Otherwise, it would have to be assumed that a neuron on the left side and the corresponding cell on the right side, as, for example, Mauthner's neuron, must each possess its own right-left specificity. Although this is entirely possible, supporting evidence for it is lacking. Asymmetrical associations might also be formed by a single neuron, provided that there was a temporal delay involving a shift of differentiation between the formation of synapses on one side and on the other.

To date, little more than a beginning has been made in attacking the many problems relating to the establishment of neuronal specificity. It is only in a few instances that we have begun to obtain some idea of how it arises and how it determines the properties of the developed system. With regard to the underlying chemistry of the phenomena, no direct evidence whatever is available, although some tentative suggestions have been proposed (Weiss, 1947) regarding its possible nature [illegible]
tiple [illegible]
syster[illegible]
[illegible] specification. The studies most relevant at present [illegible]
sis of antigen-antib[illegible]
tiple specificities. Thus the methods of immunochemistry would seem to hold promise for future analysis.

Other problems remain concerning the manner in which [illegible]

tegumentum must now be considered subject to local chemical specification in so far as their sensory innervation is subject to local sign properties. Formerly it was thought that the learning and conditioning process was responsible for all such refined functional differentiation of the nervous system, but it has now become evident that it is built into the system by inherent developmental processes which depend on local differentiation of the tissues involved.

Neuron specificity is presumed to arise by processes of cell differentiation similar to those that cause developmental differentiation in other tissues. In the nervous system, however, the result frequently is more subtle and involves no visible distinctions. The over-all process of differentiation is presumed to follow a treelike pattern, as a rule, with the gross subdivisions being set off first and these, in turn, successively subdivided to produce increasing refinement. As a result, the chemical properties of the individual neuron elements, as finally determined, are not haphazardly arranged but exhibit systematic familial relationships reflecting rather closely the functional relations.

Many neurons form a wide variety of functional associations. For example, some of the second-order vestibular cells send an ascending axon branch into the motor ocular nuclei and also a descending branch that fires other cell types at various lower levels of the bulb and cord. Similarly, many sensory fibers form a variety of functional associations in different segments of the cord and medulla. Thus, although the specificity of a neuron determines what other particular cells it is able to excite, this selectivity is not restricted to a single cell type. It may encompass a variety of neurons, but always according to a precise plan. Along with its excitatory associations, a neuron may possibly also maintain an array of inhibitory relations with antagonistic cells. In any case, the developed pattern of biochemical associations within the centers is of an extremely complex and delicate design. It is in neurogenesis that the developmental processes attain their peak of refinement and complexity.

The induction of specificity throughout a neuron merely by the end-organ connection of its axon tip (Weiss, 1941, 1942; Miner, 1950) points to some type of chain reaction which starts at the fiber tip and passes over the whole extent of the nerve cell. That induction can be accomplished under such conditions may be significant with reference to the problem of embryonic induction in general. This specification of nerve cells through their terminal contacts is to be contrasted with specification achieved through direct self-differentiation of the cell bodies themselves within the ganglia and central

gray. The final specificity of a given neuron in many instances probably represents a complex product of the two processes.

The attainment of differentiation through terminal contacts with tissues far distant from the cell bodies releases the nervous system in some instances from certain of the limitations of differentiation to which other tissues are subject. In the ventral columns of the cord, for example, where there emerge in the limb segments as many different types of neurons as there are muscles in the limbs, it becomes possible for motor cells of quite diverse specificity to lie adjacent to and erratically intermingled with one another because their specification is acquired via axonal contacts with the musculature.

Thus far the evidence indicates that the pattern of specificity on either side of the sagittal mid-plane is a mirror image of that on the other. The neurons of the biceps brachialis, of the dorsal quadrant of the retina, or of the V sensory nucleus, for example, are apparently alike on right and left sides. If this holds throughout, it means that any given nerve cell could maintain only symmetrical relations on each side of the mid-line. Wherever asymmetrical excitation is initiated from unilateral stimulation, separate sets of neurons must be present to carry the impulses across the mid-line. Otherwise, it would have to be assumed that a neuron on the left side and the corresponding cell on the right side, as, for example, Mauthner's neuron, must each possess its own right-left specificity. Although this is entirely possible, supporting evidence for it is lacking. Asymmetrical associations might also be formed by a single neuron, provided that there was a temporal delay involving a shift of differentiation between the formation of synapses on one side and on the other.

To date, little more than a beginning has been made in attacking the many problems relating to the establishment of neuronal specificity. It is *only* in a few instances that we have begun to obtain some idea of how it arises and how it determines the properties of the developed system. With regard to the underlying chemistry of the phenomena, no direct evidence whatever is available, although some tentative suggestions have been proposed (Weiss, 1947) regarding its possible nature and *operation*. The tremendous range and multiple dimensions [illegible]
system favor s[illegible]
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Others problems remain concerning the manner in which neuronal

specificity influences function. Although it is clear that basic functional selectivity is directly determined by the specification of the neurons, the exact way in which this is brought about is still in question. Possibly the primary influence of neuronal specification is upon the physiological processes of excitation and conduction (Weiss, 1936). It is also conceivable that the primary effect may be upon the growth processes to cause formation of specific structural associations (Sperry, 1950). It is equally plausible that both the foregoing types of influence may be operative either separately or in some combination yet unsuspected.

Specific nerve energies of the sort conceived by Hering (1913) are not ruled out merely because their presence is not revealed by electronic methods. The foregoing evidence that neurons mediating different sensations actually differ in their chemical constitution invites renewed consideration of the possibility that their impulses also differ in quality and that this partly conditions the pattern of spread through the centers. Since the days of Hering little advance has been made in the understanding of the neural basis of sensory qualities, and it remains as difficult as ever to account for different qualities of sensation with only homogeneous impulses routed over different pathways.

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